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Escape Behaviour and Use of Cover by Young-of-the-Year Brook Trout, *Salvelinus fontinalis*

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The distance between young-of-the-year (YOY) brook trout (*Salvelinus fontinalis*) and an approaching observer when the trout first fled (reactive distance) was significantly shorter in areas of high cover than in areas of low cover. Reactive distance was negatively correlated with foraging rate in one stream but not in another. These results provide some support for a recent economic model of escape behaviour. Three measures of willingness to take risks while foraging, reactive distance, latency to forage after a disturbance, and use of foraging sites with overhead cover, increased with increasing body size of YOY brook trout. We suggest that stream-resident salmonids become more wary with increasing size because the relative benefits of growing quickly, and hence taking risks while foraging, decrease with increasing body size.

La distance à laquelle l'omble de fontaine (*Salvelinus fontinalis*) de moins d'un an s'est enfui à l'approche d'un observateur (distance de réaction) était nettement plus courte aux endroits à couverture élevée qu'aux endroits à faible couverture. La distance de réaction était négativement corrélée au taux de recherche de nourriture dans un cours d'eau mais ce n'était pas le cas dans un autre bassin. Ces résultats viennent en partie étayer un récent modèle du comportement de fuite. Trois valeurs du consentement à prendre des risques pendant la recherche de nourriture, soit la distance de réaction, le temps de latence de recherche de nourriture après un dérangement et l'utilisation de sites de recherche de nourriture à couverture élevée, ont augmenté en fonction d'un accroissement de la taille corporelle des ombles de fontaine de moins d'un an. Les auteurs formulent la conclusion qu'une plus grande méfiance accompagne l'accroissement de la taille des salmonidés vivant dans des cours d'eau car les avantages relatifs d'une croissance rapide, et donc de la prise de risques pendant la recherche de nourriture, diminuent avec une augmentation de la taille corporelle.

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Juvenile salmonids in streams are sensitive to overhead movements and usually hide under cover when approached by observers (Hoar 1958; Chapman and Bjornn 1969). This is presumably an antipredator response, as several species of birds are effective predators of juvenile salmonids (White 1939; Hoar 1958; Elson 1962). However, there is a cost associated with this escape behaviour; after fleeing the fish remain under cover and do not forage for a few seconds or even minutes (pers. obs.). Salmonids can decrease their risk of aerial predation by selecting foraging sites with overhead cover (Hoar 1958), but using cover may be costly in terms of a lower foraging efficiency (Wilzbach 1985; Wilzbach et al. 1986). If juvenile salmonids are indeed energy maximizers (Dill et al. 1981), they must trade off the benefits of feeding frequently and growing quickly with the risk of being preyed upon (e.g. Dill and Fraser 1984).

Ydenberg and Dill (1986) considered how an animal might balance these conflicting demands. They used an economic model to predict the distance (reactive distance) between a predator and its prey at which the prey should initially flee from the approaching predator. From the point of view of the potential prey, risk of predation presumably increases as distance to the predator decreases so the cost of remaining (i.e. not fleeing) also increases. At the same time, the cost of fleeting (e.g. lost foraging opportunities) decreases as reactive distance decreases. The optimal reactive distance is defined by the intersection of the two cost curves (see their fig. 1). A key assumption of their model is that animals often detect predators long before they actually flee, so reactive distance is the result of an economic "decision" rather than perceptual constraints such as visual acuity or vigilance of the prey species.

In this study we investigated how young-of-the-year (YOY) brook trout (*Salvelinus fontinalis*) balance foraging behaviour with risk of predation. We assumed that risk of predation increases with distance from cover and, therefore, predicted an increase in reactive distance for brook trout that are farther from overhead cover. We also assumed that the cost of fleeing is proportional to foraging rate because a fish that is foraging frequently has more to lose by fleeing than a fish that is foraging less frequently. Alternatively, one might assume that foraging rate is inversely proportional to hunger and that hungrier fish should take greater risks when foraging (e.g. Milinski and Heller 1978). However, juvenile salmonids in streams rarely have full stomachs (Jenkins et al. 1970; Johnson and Johnson 1981; pers. obs.) and forage throughout the daylight hours. Therefore, we predicted a negative correlation between foraging rate and reactive distance.

In 1984, we noticed that YOY brook trout seemed to become more wary with increasing size. A second objective of the study was to investigate the effects of body size on three measures of wariness: reactive distance to the approach of an observer, latency to forage after a disturbance, and use of foraging sites with overhead cover.

Methods

YOY brook trout were observed in three streams east of Guelph, Ontario: Black Creek, a third-order stream that flows into the West Branch of the Credit River (43°38′N, 79°59′W); an unnamed first-order stream, hereafter the "Meadow stream," that flows into the Credit River upstream of the Forks of the Credit (43°49′N, 80°01′W); and the north branch of the Credit River downstream of Highway 24 (43°50′N, 80°01′W), a fourth-order stream. Each study site was approximately 50 m long.

Distance to Cover and Reactive Distance

The effect of distance to cover on reactive distance was investigated in the Meadow stream in 1986. Discrete areas of high and low cover were selected before testing. Areas of low cover were characterized by a light-coloured sand substrate with little aquatic vegetation. Areas of high cover contained clumps of aquatic macrophytes (*Nasturtium officinale* and *Veronica anagallis-aquatica*) that covered more than 50% of the substrate.

The reactive distances of 10 fish were measured in high and low cover areas on each of two days: May 12 and May 24, 1986. Fish were initially sighted from downstream using 7 × 35 binoculars. The observer approached a fish from downstream until it was just visible by the naked eye, usually about 4 m away. The length of the fish was estimated and the reaction distance to the approach of the observer from a downstream direction was determined. Even fish in high-cover areas occupied sites in the open and could be seen through binoculars from 5 to 7 m downstream. We presume that all fish were equally able to see the approach of the observer. The actual hiding place could not be determined during the tests, so we used our a priori assessment of amount of cover to categorize fish with respect to distance to a hiding place. This assessment was tested by measuring the distance to the nearest patch of cover greater than 8 cm in diameter, after the disturbance. Cover was defined as anything offering concealment from above (Bachman 1984) and included aquatic plants, logs, rocks, and riffles on the surface of the water. All types of cover are not necessarily equivalent in terms of protection from predators. However, in the absence of a method for ranking different types of cover, we assumed all types were equal.

Foraging Rate, Body Size, and Reactive Distance

Fish were observed in two streams at approximately monthly intervals over the summer of 1985: Black Creek — May 22, June 18, July 23, and August 14; Meadow stream — May 2, May 23, June 20, and August 1. Ten fish were observed on each day, with the exception of August 14, when only 9 fish were observed. The average current velocities 5 cm above the substrate were low (Black Creek = 16 cm/s, Meadow stream = 7 cm/s) and water depths were shallow (Black Creek = 26 cm, Meadow stream 19 cm) at both sites, so fish could be observed in most areas of the study sites.

All behavioural data were collected by an observer who sat motionless about 2 m downstream of the fish under observation (test fish). The observer waited for at least 15 min after sitting down to allow fish to reoccupy their sites and resume foraging in an undisturbed manner. Only fish that had occupied a single site for at least 10 min and were actively foraging were observed. The number of foraging attempts in a 5-min period was recorded on a portable tape recorder. A foraging attempt was defined as any quick movement to intercept a potential food item, whether successful or not. The fork length of the test fish was estimated to the nearest 0.5 cm by comparing the fish to landmarks near the foraging site, which were measured after the observation. Immediately after the 5-min observation a second observer (intruder) approached the test fish at a slow walk (~0.5 m/s). The distance between this observer and fish at the time the flight response was initiated (reactive distance) was measured. The direction of approach by the intruder could not be standardized in all cases because of stream bank vegetation and the position of the first observer. Therefore, the direction of approach was chosen to give the test fish the clearest view of the approaching intruder and to prevent casting shadows that might startle the fish. The test fish was approached from either the side (3 or 9 o'clock) or downstream (6 o'clock). Direction of approach had no effect on reactive distance ($F = 0.12$, $p = 0.73$). The observer moved upstream a few metres after each observation to avoid reobserving the same fish and to prevent habituation of the fish to the intruder. The 10 observations per day were spread over about a 6-h period between 10 a.m. and 4 p.m.

Latency to Forage, Use of Cover, and Body Size

The effect of body size on the time to resume foraging after a disturbance (latency to forage) was examined in Black Creek and Meadow stream in 1986. A 1-m^2 quadrat was placed in an area where YOY brook trout were known to forage from a stationary position (holding position). Areas of extremely low or high cover were avoided. An observer sat motionless 1 m downstream of the quadrat for 30 min to allow the fish to reoccupy their foraging positions. After the 30 min, the length of each fish that was holding position in the quadrat was noted. The observer then disturbed the fish by standing, walking to the downstream edge of the quadrat, and touching the middle of the quadrat with his hand. The observer resumed sitting 1 m downstream and recorded the time required for 50% of the original number of fish to resume holding position in the quadrat. Initially, fish did not always return to exactly the same foraging site; larger fish often held position and foraged for several minutes before reoccupying their original site. Time to hold position was used as an estimate of latency to forage because the former was easier to measure when several fish were being followed simultaneously. Foraging attempts usually commenced within a few seconds of holding position. Latency to forage was interpolated if there was an odd number of fish in the quadrat. The lengths of the first 50% (50% + 1 for odd numbers of fish) of the fish that resumed foraging were estimated to the nearest 0.5 cm. The initial number of fish in the quadrat ranged from 2 to 9 (median = 4).

Ten observations were made in Black Creek from May 13 to June 3, and 20 in Meadow stream from April 30 to July 15. Observations were distributed over the available habitat to decrease the probability of habituation and of observing the same fish twice.

Use of cover was assessed by skin-diving surveys. The diver moved upstream slowly through the study reach placing numbered stones on the locations that undisturbed YOY brook trout

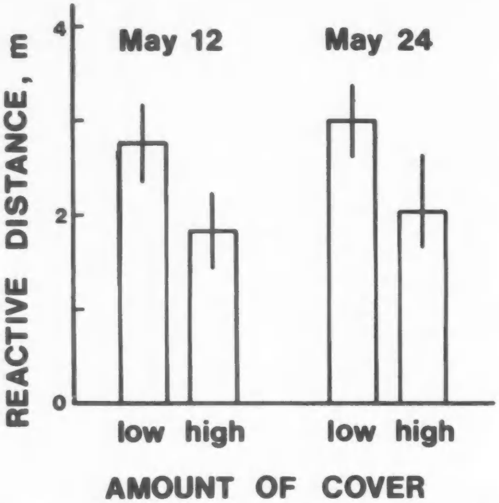

FIG. 1. Reactive distance (±95% confidence interval) of brook trout was significantly shorter ($p < 0.001$) in areas of high cover on two days in Meadow stream.

were occupying as foraging sites. On each survey the diver attempted to observe a random sample of the fish. Observations from the stream bank were used to sample shallow-water areas which were inaccessible to the skin-diver. Each foraging site was scored for the presence or absence of cover as defined above. Eleven surveys were conducted over three summers (1984−86) in Black Creek, 10 over three summers in Meadow stream, and 5 in two summers (1984 and 1986) in Credit River.

Availability of cover was assessed at locations every 0.5 m along transects across the streams. The transects were spaced regularly along the section of stream that was surveyed by the diver. Current velocity 5 cm above the substrate was measured at each point with a Teledyne−Gurley Pigmy Current Meter. Habitat availability was similar in the three years, so only the 1984 data were considered. Surveys of habitat availability were conducted on the following days: Black Creek — June 26 and August 3; Meadow stream — June 12 and July 26; Credit River — July 10 and August 14.

Results

Distance to Cover, Foraging Rate, and Reactive Distance

Reactive distance was significantly shorter in areas of high cover than in areas of low cover ($F = 23.56$, $p < 0.001$; Fig. 1). The effects of date or the interaction of date and amount of cover were not significant ($p = 0.25$ and $p > 0.95$, respectively; analysis of variance). Distance to cover was, as expected, lower in areas of high cover (mean = 14.0 cm) than in areas of low cover (mean = 68.5 cm, $p < 0.001$; t-test). There was no significant difference in the length of the fish observed in the two areas ($p > 0.50$; t-test).

Reactive distance and foraging rate were significantly negatively correlated at Black Creek but were not correlated at the Meadow stream (Fig. 2). Although there may appear to be several outliers in Fig. 2a, omitting the four fish with the highest foraging rates or the fish with the greatest reactive distance did not greatly affect the correlation coefficient. To ensure that the correlation at Black Creek was not the result of body size (see below), we statistically controlled for body length. There was still a significant negative correlation between foraging rate and reactive distance at Black Creek (partial $r = -0.366$, $p < 0.01$).

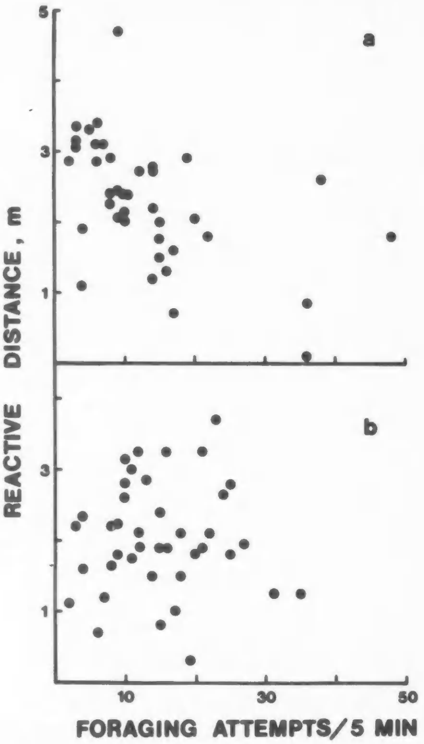

FIG. 2. Relationship between reactive distance of brook trout and number of foraging attempts per 5 min in (a) Black Creek ($r = -0.498$, $n = 39$, $p < 0.001$) and (b) the Meadow stream ($r = +0.016$, $n = 40$, $p = 0.924$).

Wariness, Use of Cover, and Body Size

Reactive distance was significantly positively correlated with fork length, even after controlling for foraging rate, at Black Creek (partial $r = 0.482$, $p < 0.01$) and Meadow stream (partial $r = 0.695$, $p < 0.01$). There were no significant differences in the slopes ($p = 0.82$) or intercepts ($p = 0.54$; analysis of covariance) of the reactive distance versus fork length regressions between the streams, so one regression line was plotted (Fig. 3). The reactive distance − body length relationship apparently could be extrapolated for much larger fish, as the reactive distance for three 200-mm fish (4.6, 6.7, and 6.9 m) compared well with the predicted value (6.3 m) from Fig. 3.

Latency to forage after a disturbance was also positively correlated with body length of the fish ($p < 0.001$; Fig. 4). This same trend was observed within each observation, as the mean length of the first 50% of the fish returning after the disturbance was smaller than the mean length before the disturbance in 19 of 25 cases when there was a difference ($p = 0.014$; sign test).

Larger YOY brook trout tended to use foraging sites that were increasingly associated with overhead cover (Fig. 5a). A

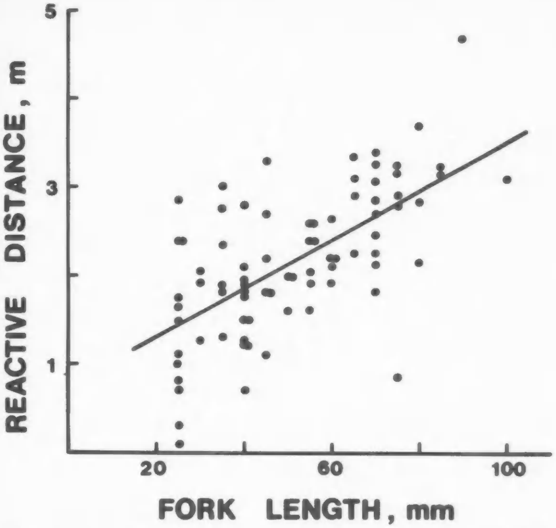

FIG. 3. Relationship between reactive distance of brook trout and fork length ($Y = 0.0277X + 0.741$; $r^2 = 0.409$, $n = 79$, $p < 0.001$). Data for Black Creek and the Meadow stream are combined.

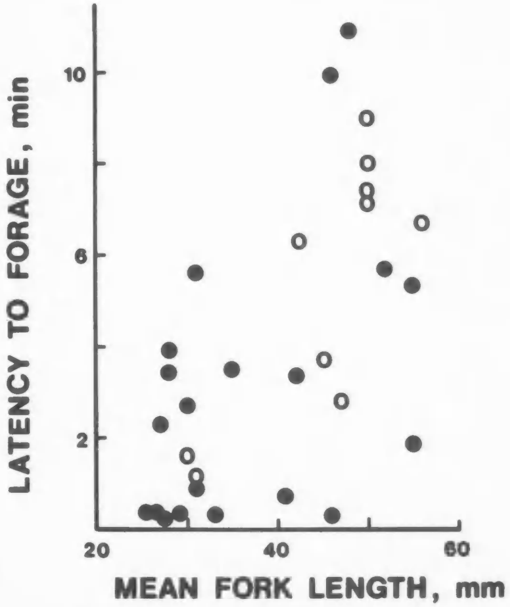

FIG. 4. Relationship between time for 50% of brook trout in a 1-m^2 quadrat to reoccupy their foraging sites after a disturbance and their mean body length ($r = 0.616$, $n = 30$, $p < 0.001$). ●, Meadow stream; ○, Black Creek.

three-way contingency table analysis indicated that percent use of cover increased significantly with body size ($\chi^2 = 38.72$, df = 6, $p < 0.001$). There were also significant differences among streams in percent use of cover ($\chi^2 = 7.29$, df = 2, $p = 0.026$), but there was no interaction between stream and body size ($\chi^2 = 10.24$, df = 12, $p = 0.59$). The availability

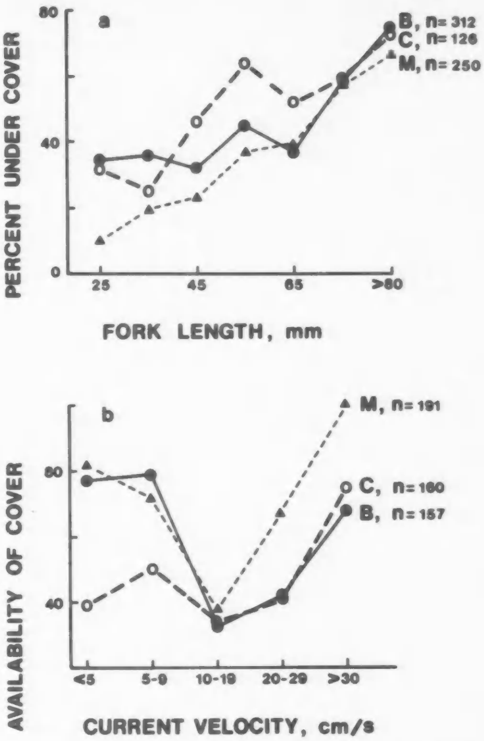

FIG. 5. (a) Relationship between percent of foraging sites with overhead cover and fork length of brook trout in three streams (n = sample size; B = Black Creek, M = Meadow stream, C = Credit River). (b) Percent availability of cover as a function of current velocity 5 cm above substrate at points along regularly spaced transects across the streams.

of cover initially decreased with increasing current velocity, reached a minimum at 10−19 cm/s, and then increased in faster water (Fig. 5b). YOY brook trout were generally found in current velocities less than 10 cm/s when less than 30 mm in length, gradually moved into faster water as they grew larger, but rarely used current velocities faster than 30 cm/s (J. W. A. Grant, unpubl. data). Therefore, the increasing use of cover with increasing body size did not appear to be a function of the availability of cover at different current velocities and may indicate a real size-dependent preference for overhead cover.

Discussion

Distance to Cover, Foraging Rate, and Reactive Distance

Fish closer to cover had shorter reactive distances (Fig. 1). The approaching intruder was presumably a less threatening stimulus to the fish closer to cover, resulting in a shorter reactive distance and more time devoted to foraging. This is apparently one of the first studies to quantify an effect of distance to cover on reactive distance (Ydenberg and Dill 1986) and is consistent with their economic model of escape behaviour. YOY brook trout appear to adjust their reactive distance based on risk of predation.

Reactive distance was negatively correlated with foraging rate only at Black Creek. However, was this negative correlation the result of an economic decision or a perceptual constraint? Perhaps fish that were foraging more frequently were less vigilant (e.g. Milinski 1984) and hence had lower reactive distances than fish that were foraging less frequently. We cannot rule out this alternate explanation but think it is unlikely because even actively foraging fish spend most of their time holding position over a central focal point, scanning the water column for drifting prey organisms (McNicol et al. 1985). While scanning for prey, fish can also be watching for conspecific intruders or predators. We presume that vigilance would only be compromised during the actual feeding foray, which takes about 1 s (J. W. A. Grant, unpubl. data). Because most of the decrease in reactive distance in Black Creek occurred over a range of 1−4 foraging attempts/min, foraging rate should have only a minor effect on vigilance.

We cannot explain the difference in the reactive distance versus foraging rate relationship between the two streams. The only apparent difference between the two streams was in the amount of food; drift density was higher in Black Creek (J. W. A. Grant, unpubl. data). However, there was no difference in mean foraging rate ($t = 0.76$, $p > 0.40$) or reactive distance ($t = 1.35$, $p > 0.10$) between the two streams. These results provide only equivocal support for Ydenberg and Dill's (1986) model. It is possible that measuring foraging rate for only 5 min may not have been a reliable estimate of the costs of fleeing.

Wariness, Use of Cover, and Body Size

Reactive distance, latency to forage, and use of cover all increased with body size, suggesting a general increase in wariness of YOY brook trout with increasing size. We use "wariness" as an inverse of the fish's willingness to take risks while foraging. The trend of increasing wariness with body size seemed to hold for older/larger fish, as individuals more than 1 yr old generally occupied foraging sites under cover (Gibson 1966; pers. obs.) and reactive distance continued to increase with body size as discussed above. The increase in reactive distance with body size could be explained by a constraint hypothesis (Ydenberg and Dill 1986) because visual acuity increases with fish size (e.g. Li et al. 1985). However, the latency to forage data suggests that the increase in wariness with body size is not simply a function of perceptual capabilities. We can also rule out spurious correlations between wariness and some factor that changes seasonally because smaller fish resumed foraging after a disturbance even within an observation. We also do not think that selection by predators against less wary fish could by itself explain the data in Fig. 3, 4, and 5. For example, the increase in reactive distance in Fig. 3 was significant and yet there was no change in the variance in reactive distance over the first summer of life (Kendall's $\tau = 0.143$, $n = 8$, $p \gg 0.05$). One would expect a decrease in variance with time if the increase in reactive distance was caused by selection by predators.

We suggest that there is a direct relationship between reactive distance and latency to forage; fish that have greater reactive distances probably also take longer to return after a disturbance. There is presumably a trade-off between time available to forage and protection from predators that is associated with reactive distance or latency to forage. It is perhaps less obvious how use of cover relates to the first two measures of wariness. Fish holding position under cover may have a lower foraging efficiency than fish in the open (Wilzbach 1985; Wilzbach et al. 1986). The percent of prey captured by *Salmo clarki* decreases with decreasing light levels (Wilzbach et al. 1986), probably because reactive distance to prey is impaired (Vinyard and O'Brien 1976). Therefore, if a fish's complete foraging zone is in the shade, its foraging efficiency will decrease. However, a fish that holds position in the shade and forages in the open may have a visual advantage over fish that hold position in the open (Helfman 1981). Wilzbach (1985) and Wilzbach et al. (1986) suggested that cover can decrease foraging efficiency in a second manner; irregularities in the substrate and objects upstream of the fish may obstruct the fish's field of vision. Therefore, there may also be a trade-off between foraging efficiency and protection from predators associated with using overhead cover, just as there is a trade-off between time available to forage and either reactive distance or latency to forage after a disturbance. We consider the three measures of wariness to be interchangeable with respect to the predictions of Ydenberg and Dill's model.

TABLE 1. Size- or age-dependent changes in wariness and use of cover by stream-dwelling salmonids.

Observation	Source
YOY *Salmo salar* moved from shallow gravel riffles to areas with shelter with increasing size	McCrimmon 1954
Reactive distance of brook trout increased with age	Keenleyside 1962
After a disturbance, smaller *Oncorhynchus kisutch* left fright huddles and began feeding before larger fish	Mason and Chapman 1965
Yearling *Salmo gairdneri* preferred areas with overhead cover, while YOY showed no preference	McCrimmon and Kwain 1966
YOY *Salmo trutta* are distributed in the open, yearlings move under cover with increasing size, and larger fish are rarely seen	Le Cren, cited in Chapman and Bjornn 1969
Distance to cover increased with age for brook trout and *Salmo clarki*	Griffith 1972
Yearling brook trout and *Salmo gairdneri* were found more frequently under cover compared with YOY at one site; no significant differences at a second site	Cunjak and Green 1983
Percentage of time spent under cover increased with age for *Salmo trutta*	Bachman 1984
Yearling *Oncorhynchus tshawytscha* were larger in covered than in uncovered sections of an artificial stream	Brusven et al. 1986
Older (>1) brook trout and *Salmo trutta* were found more frequently under cover than YOY in three of four streams in the summer; no significant differences in the winter	Cunjak and Power 1986
Larger *Salmo salar* parr were more wary than smaller parr	Morantz et al. 1987

A number of studies have noted an increase in the wariness or use of cover of stream-resident salmonids with increasing size or age (Table 1; Griffith 1972 is an exception). Similarly, large salmonids appear to prefer overhead cover (Butler and

Hawthorne 1968; Gibson 1978; Devore and White 1978) whereas YOY may even avoid cover (Ruggles 1966). However, we should caution that none of the field studies of habitat use in Table 1 adequately documented habitat availability, so one cannot conclude that preferences for cover necessarily increased with body size. As well, we do not suggest that size-specific changes in wariness are necessarily the only explanation for all of the observations. For example, Brusven et al. (1986) suggested that larger fish were found in covered rather than uncovered areas of an artificial stream because of intraspecific competition for preferred sites under cover. Despite these reservations, Table 1 suggests that the increase in wariness with body size noted in our study for brook trout may be a general pattern for stream-resident salmonids.

Why should wariness increase with body size? The mortality rate of YOY salmonids is highest just after emergence and decreases with increasing body size (Latta 1962; Le Cren 1973; Elliott 1985). Most of this early mortality is thought to be the result of starvation rather than predation (Latta 1969; Elliott 1986). Fasting endurance increases with body mass (M) for stream salmonids (Bagenal 1969; Latta 1969; Elliott 1984) because body reserves are typically proportional to $M^{1.0}$ whereas metabolic rate is proportional to $M^{0.75}$ (Calder 1984). If the probability of starvation decreases with increasing body size, then the costs of fleeing (sensu Ydenberg and Dill 1986) should also decrease with increasing body size. In addition, reproductive value, the age-specific expectation of future offspring, typically increases with age until the age of maturity and then declines (Pianka 1983), but may continue to increase with body size past the age of maturity for salmonids because they have indeterminate growth and fecundity increases with body mass (see data in Elliott 1984, 1985). YOY salmonids have low reproductive value because their probability of surviving to reproduce is low. Therefore, YOY salmonids should benefit more from growing quickly than will larger fish that are closer to or are of reproductive size; i.e. a fish below reproductive size has more to lose by not growing than a fish of reproductive size. We suggest that salmonids should take greater risks while foraging when smaller or younger than when larger or older in order to grow as quickly as possible.

Two other factors may also be responsible for the increase in wariness with body size. Risk of predation in relation to body size is a major unknown factor, but it seems likely that larger trout may be more visible and, hence, susceptible to predation by birds. Secondly, dominance status is positively correlated with body size (J. W. A. Grant, unpubl. data). Smaller fish resumed foraging more quickly after a disturbance than larger fish, and often occupied the foraging sites of larger fish until displaced. Smaller fish may have been taking advantage of lower competition following the disturbance to increase their foraging rate, as Barnard (1980) reported for house sparrows (*Passer domesticus*). Both of these suggestions are consistent with the economic model of escape behaviour.

The apparent increase in use of cover with increasing body size suggests that the addition of cover, recommended by stream improvement handbooks (e.g. Department of Fisheries and Oceans 1980), may have different effects on different sizes of fish. Large cover structures appear to be beneficial for larger YOY or older fish (Boussu 1954). However, in our study, brook trout less than 30 mm in length avoided overhead cover when selecting foraging sites (Fig. 5). Even in areas of dense macrophytes, small brook trout were usually found at the downstream end of small open areas and appeared to be attempting to maximize their field of vision for foraging. Smaller YOY might avoid large cover structures entirely or forage less efficiently while under them (Wilzbach 1985; Wilzbach et al. 1986).

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Emigration and Production of Hatchery Coho Salmon (*Oncorhynchus kisutch*) Stocked in Streams Draining an Old-growth and a Clear-cut Watershed

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Downstream movement of coho salmon fry (*Oncorhynchus kisutch*) stocked in old-growth and clear-cut watersheds occurred in three phases: (1) a brief period of heavy emigration immediately after stocking, (2) relatively little movement throughout most of the summer, and (3) intermittent heavy emigration during early autumn freshets. Coho emigrated whenever a streamflow change $\geq 3\% \cdot d^{-1}$ occurred, but movement nearly ceased at flows above a certain level. Temperature changes were less important than discharge in triggering movement. When high densities were stocked, emigrant fry were smaller than residents. When low densities were stocked, emigration after the initial pulse of downstream movement was generally lower and there were no size differences between emigrants and residents. Production in the clear-cut was greater than in the old-growth watershed. Proportionately fewer fish emigrated from the old-growth stream, but when population densities were high, mortality in the old-growth exceeded the clear-cut. Greater emigration from the clear-cut site was possibly related to a scarcity of pools. Although the old-growth stream possessed better rearing habitat, less food may have been available, as suggested by gross photosynthesis rates 50% lower than in the clear-cut stream. Coho production therefore appeared to be most strongly influenced by trophic conditions, while volitional residency was most strongly influenced by habitat quality.

Les déplacements vers l'aval d'alevins de saumon (*Oncorhynchus kisutch*) ensemencés dans des bassins versants à peuplement mûr ou faisant l'objet d'une coupe rase se sont effectués en trois phases : 1) une brève période d'émigration marquée immédiatement après l'ensemencement, 2) relativement peu de déplacements pendant presque tout le reste de l'été et 3) une émigration marquée intermittente pendant les crues du début de l'automne. Les cohos ont émigré quand le débit variait de $\geqslant 3\ \% \cdot j^{-1}$ mais les déplacements ont presque cessé à des débits supérieurs à un certain volume. Les variations de la température étaient moins importantes que le débit dans le déclenchement des migrations. Quand l'ensemencement avait lieu à des densités élevées, les alevins émigrants étaient plus petits que les résidents. Par contre, quand les alevins étaient ensemencés à de faibles densités, l'émigration après la pointe initiale de déplacement vers l'aval était généralement réduite et il n'y avait aucune différence de taille entre les émigrants et les résidents. La production dans le bassin versant faisant l'objet d'une coupe rase était plus élevée que dans le bassin versant à peuplement mûr. Un nombre proportionnel moins élevé de poissons ont émigré du cours d'eau à peuplement mûr mais quand les densités étaient élevées, la mortalité à cet emplacement dépassait celle observée dans le bassin versant faisant l'objet d'une coupe rase où le taux d'émigration plus élevé était possiblement lié à la rareté des mares. Quoique le bassin versant à peuplement mûr offrait de meilleures zones de croissance, il semblait contenir moins de nourriture comme le portent à croire des taux de photosynthèse bruts qui sont 50 % moins élevés que ceux observés dans le bassin versant faisant l'objet d'une coupe rase. Les niveaux trophiques semblent donc fortement influer sur la production de cohos tandis que le séjour volontaire était plus fortement touché par la qualité de l'habitat.

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Large numbers of coho salmon (*Oncorhynchus kisutch*) frequently emigrate from natal streams after emerging from spawning redds (Chapman 1962). Downstream movement of these fry tends to be greatest immediately after emergence, but often continues at a lower level throughout the summer and autumn. The timing and magnitude of emigration are driven by environmental factors whose influences are known from only a few case studies and which apparently are not consistent from stream to stream. Emigration was originally believed to be the result of accidental displacement of fish that failed to find hiding places among the stream's stony substrate at night and were unable to maintain a visually oriented feeding station (Hoar 1958). However, recent evidence has pointed to the existence of a wide variety of environmental cues that facilitate increased downstream movement. The nature and importance of such cues has received widespread attention because emigration coho from nursery streams may account for more than half of the initial fry population. The survival to maturity of presmolt emigrants that enter salt water is believed to be very low (Mason 1975; Crone and Bond 1976; Washington 1981; Holtby and Hartman 1982), although some emigrants establish themselves in lower stream reaches if suitable habitat can be located (Chapman 1962; Mason 1969; Scrivener and Andersen 1984).

The factors influencing downstream movement of coho fry include both physical and biological conditions. Chapman (1962) noted that initial coho fry densities may exceed a stream's carrying capacity, and aggressive behavior among individuals competing for feeding territories results in downstream displacement of subordinates. Mason and Chapman (1965) demonstrated a positive correlation between level of aggression and fry emigration from artificial stream channels, and Chapman (1962) found that fry emigration rate was proportional to the initial population density in three Oregon streams. However, in Carnation Creek, a relatively short Vancouver Island stream prone to severe freshets, fry movements were principally associated with freshets and most of the fish leaving the vicinity of spawning areas were transported completely out of the stream system (Hartman et al. 1982). In addition to being associated with population pressure and discharge peaks, coho fry movements have coincided with lunar phases (Mason 1975), temperature change (Keenleyside and Hoar 1954; Chapman and Bjornn 1969; Hartman et al. 1982), and turbidity increase (Noggle 1978; Berg 1982; Sigler et al. 1984). Downstream movements may also result from volitional attempts to locate suitable overwintering habitat (Bustard and Narver 1975a, 1975b; Peterson 1982; Tschaplinski and Hartman 1983).

Research to date has focused on wild populations. However, stocking of hatchery coho fry has become common, especially in watersheds where escapement of adult fish is below the number needed to fully seed the system or where habitat above barriers is perceived to be underutilized. It is unknown if hatchery fry exhibit the same emigration patterns as wild fish, or if their movement is triggered by similar environmental cues. The impact of logging on emigration and production is also not fully understood. Hartman et al. (1982) found that clear-cut logging along Carnation Creek caused increased water temperatures and earlier emergence of coho from spawning gravel in the spring. As a result, emigration began earlier in the year, but resident fry gained the advantage of a longer growing season. Higher salmonid production rates in other open-canopied streams in the Pacific Northwest have been attributed to a variety of causes, including increased autotrophic production resulting from more sunlight reaching the channel (Murphy et al. 1981; Weber 1981; Hawkins et al. 1983; Walter 1984; Duncan and Brusven 1985), increased water temperature (Narver 1972; Osborn 1980), and increased foraging efficiency (Wilzbach 1985). The objective of our study was to identify the important environmental clues triggering the emigration of hatchery coho fry stocked in an old-growth coniferous forested watershed and a clear-cut watershed and to relate the patterns of downstream movement to differences in summer production between the two sites.

Study Area

The study was conducted in the headwaters of the Deschutes River system (46°43′N, 122°23′W), which drains the foothills of the Cascade Mountains of western Washington. West Fork Creek (the old-growth site) drains an 850-ha watershed consisting of undisturbed coniferous forest dominated by Douglas-fir, western hemlock, and western red cedar. Most of the watershed of the adjacent 255-ha upper Deschutes River study site (the clear-cut site) was logged in 1974−75 and replanted with Douglas-fir in 1975−76. The riparian zone of this stream is dominated by small willows, red alder, salmonberry, and herbaceous vegetation. Differences in discharge between the two streams are less than would be expected based on differences in drainage area due to greater precipitation in the clear-cut watershed. Mean annual discharges at the clear-cut site were 0.65 $m^3 \cdot s^{-1}$ in 1982 and 0.64 $m^3 \cdot s^{-1}$ in 1983. Corresponding values for the old-growth site were 1.12 $m^3 \cdot s^{-1}$ in 1982 and 1.11 $m^3 \cdot s^{-1}$ in 1983.

TABLE 1. Habitat composition of the study streams, following the classification system of Bisson et al. (1982).

	Deschutes River (clear-cut)	West Fork Creek (old-growth)
Midsummer dimensions		
Length (m)	377	451
Area (m^2)	872	1502
Volume (m^3)	120	277
Pool habitat (% total area)		
Backwater	5.6	6.0
Scour	16.1	25.7
Plunge	8.7	23.9
Secondary channel	2.0	2.1
Dammed	0	1.1
Total pool habitat	32.4	58.8
Riffle habitat (% total area)		
Low gradient riffles	23.3	14.7
Rapids	3.3	8.1
Cascades	41.0	18.4
Total riffle habitat	67.6	41.2

Reaches of stream chosen for study were bounded by a fish trap on their downstream end and a falls impassable to upstream movement by salmon fry at the upstream end. The clear-cut study reach was shorter and possessed a smaller stream area and volume than the old-growth reach (Table 1), and it also had a slightly higher gradient. The clear-cut stream channel was nearly devoid of large woody debris, having been cleaned following logging. As a result, the relative abundance of pool habitat in the clear-cut stream was much lower than in the old-growth stream, where the channel was heavily loaded with woody debris. At midsummer stream flows the frequency of pool habitat in the clear-cut site was approximately half that of the old-growth stream. The remainder of the surface areas at both sites consisted of various types of riffles.

Only two fish species resided in the streams naturally, cutthroat trout (*Salmo clarki*) and shorthead sculpin (*Cottus confusus*). Average midsummer biomasses and densities of trout on an areal basis during the three years prior to the experiment were 1.8 times and 2.5 times greater, respectively, in the clear-cut site than in the old-growth site (Bisson and Sedell 1984). Likewise, sculpin biomasses and densities prior to the experiment were highest in the clear-cut watershed, being 3.0 times and 5.3 times greater, respectively, than in the old-growth stream. Neither resident species exhibited migratory tendencies, and over the period of study, very few cutthroat or scuplin entered the fish traps. Therefore, only the emigration patterns of coho are reported here. We do not know to what extent the downstream movement and production of coho in the two watersheds were affected by differences in the abundance of resident trout and sculpin populations between the old-growth and clear-cut watersheds.

Care should be taken in extrapolating the results of this study in both space and time. Conditions in the streams reflected watershed history, but we studied only single examples of old-growth and clear-cut headwater tributaries. While the watersheds were adjacent to each other, they were not replicates, and the two years in which emigration was monitored were hydrologically dissimilar. For this reason, the results are presented as case studies of two different watersheds in two different years. These limitations must be considered when applying the results of this study to other drainages.

Methods

Coho salmon fry from the Puyallup River Salmon Hatchery were planted in the study streams on June 15, 1982, and again on May 5, 1983. The difference in stocking time and fish size was dictated by the timing of fry availability at the hatchery. In 1982 the initial stocking density was twice the normal stocking rate used by the Washington Department of Fisheries; in 1983, each stream was stocked according to standard procedures, which were based on the standing stocks of wild coho in Oregon coastal streams (Chapman 1965). The two different initial densities were chosen to evaluate the effect of population pressure on survival, growth, and emigration. In 1982, average size at release was 1.88 g whereas in 1983 the fry were much smaller, averaging 0.75 g at stocking. No differences in size or condition factor existed between the fish released into the two study streams, based on subsamples of fry planted at each site.

Movement of fish from the streams was monitored with permanently installed fish traps at the downstream end of each study reach. The fish traps were constructed of steel mesh screening bolted to concrete footings which were, in turn, anchored to bedrock. A single opening in each screen led to a baffled, wooden live-box. The traps were emptied daily for 4 wk immediately after stocking when emigration was heavy. When the initial emigration had subsided, the traps were emptied every 3 d, although on several occasions the traps were left as long as 7 d between checks. On three occasions during 1983, high discharge forced the lid off the live-box in the old-growth site. These occasions occurred 1, 2, and 10 d after stocking during a period of heavy downstream movement of the fish. Therefore, we estimated the number of coho that would have been expected to emigrate on those dates based on the ratio of emigration rates between the old-growth and the clear-cut sites observed the same number of days after stocking in 1982.

Coho remaining in the streams were sampled during summer and fall. Several randomly chosen examples of each habitat type (Table 1) were isolated with blocking nets and electrofished at least three times. The density of fish within individual habitats was estimated by the removal summation technique of Carle and Strub (1978). The total population of coho in the stream was determined by multiplying the average density obtained for each habitat type by the frequency of that habitat type in the study section. Biomasses were based on length−weight regressions derived from samples of 30 fish selected at random from each stream on each date. Mortality was estimated as the difference between the estimated population size and the number of downstream migrants. Emigration was expressed as the percent of the remaining stream population collected in the trap each time the trap was emptied. Such expression facilitated a more appropriate comparison of downstream movement than the absolute number of coho fry leaving the streams. Production estimates followed Chapman (1978) in which instantaneous daily rates of growth and "mortality" (i.e. actual mortality plus emigration) were assumed to be exponential. We further assumed that the distribution of coho during the first measurement interval was reflected in the relative proportions of fish observed in each habitat during the first census, i.e. all fish captured in a particular habitat had actually spent the entire measurement interval there. However, because fish were not individually marked, these assumptions were untested.

Discharge for the two streams was estimated by correlating readings on rated staff gauges with simultaneous readings on a nearby stream with a continuous flow recorder. Recording thermographs were installed in both streams prior to fry stocking and operated continuously throughout the study periods. Average daily water temperature was taken to be the mean of the daily minimum and maximum. Daily turbidity values in the two streams were measured during the study period in 1983. Automatic pump samplers composited four samples drawn at 6-h intervals in one bottle. Bottles were collected monthly and turbidity was measured with a nephelometer.

Gross photosynthesis by stream periphyton was measured approximately every 10 d using light and dark watertight rectangular chambers (66 × 31 × 8 cm) fitted with submersible recirculating pumps (Bott et al. 1978). Trays of streambed substrate (40 × 25 cm) that had been left undisturbed in the streams for at least 3 wk were placed in the two watertight chambers, which were submerged during the measurement period (0.5−2 h, depending on the rate of oxygen production). All measurements were taken between 10:30 and 14:00 PST and probably represent peak daily production rates. Light intensity immediately above the chambers was recorded every half hour during the incubation period with a dome solarimeter. Dissolved oxygen concentrations were measured with an oxygen−temperature probe that was fitted into the wall of the chamber. Gross photosynthesis was calculated from the change in dissolved oxygen concentration during the incubation period using a photosynthetic quotient of 1.2 (Wetzel and Likens 1979).

Results and Discussion

Seasonal Patterns of Emigration

During both 1982 and 1983, coho exhibited elevated emigration rates for about 2 wk after stocking; however, emigration from the clear-cut site continued at a higher rate for a slightly longer period of time (Fig. 1). In 1982, approximately 42% of the coho fry left the stream in the clear-cut in the first 14 d after stocking, while 37% moved out of the old-growth site. For 1983, these values were 69% from the clear-cut and 55% from the old-growth. During July and August 1982, emigration rates remained relatively low, usually $>1\% \cdot d^{-1}$ (Fig. 1A). Downstream movement of fish, although infrequent, was generally higher from the clear-cut than the old-growth watershed during this period although several small peaks of emigration were seen in both streams. Substantial increases in downstream movement occurred in mid-September and early October. The mid-September emigration rates were the highest observed in 1982, excluding those immediately after stocking. The emigration rates in October were somewhat lower than those of

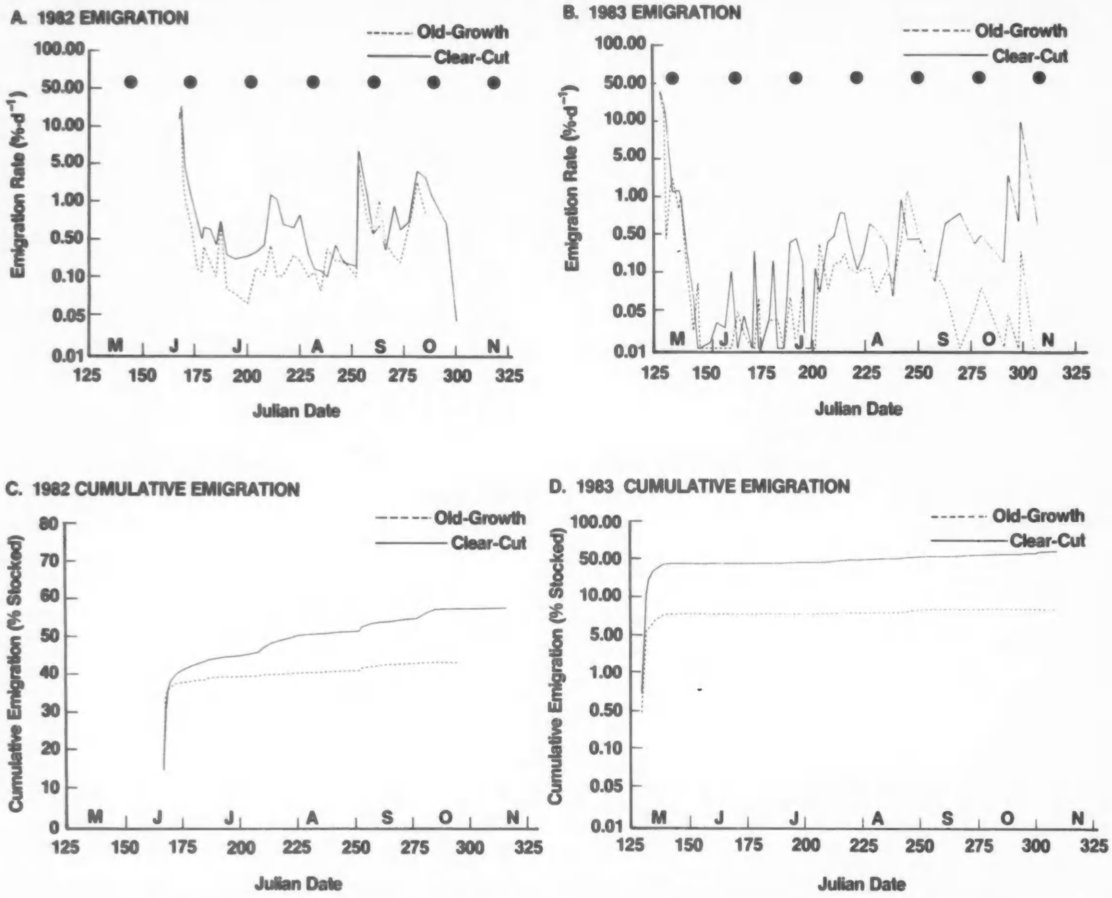

FIG. 1. (A) Emigration, as percent of resident population per day, of coho salmon from the study sites during 1982. Rates are plotted on a logarithmic scale. Solid circles indicate a new moon. (B) Coho emigration during 1983. (C) Cumulative emigration during 1982, expressed as the cumulative percent of the number of fish initially stocked in the study sites. (D) Cumulative emigration during 1983.

September but were still substantially elevated over July and August values.

Overall emigration from the two streams in summer 1983 was lower than in 1982 (Fig. 1B). Downstream movement continued to be higher from the clear-cut than the old-growth site. Very little emigration took place in June and July, with some small increases in emigration in August. Increased movement from the old-growth watershed occurred in early September, with a slight rise noted at the same time in the clear-cut site. Emigration from the old-growth site declined after the early September increase; however, coho in the clear-cut stream displayed high levels of downstream movement throughout mid- and late-October.

We separated emigration of hatchery coho fry into three distinct phases from spring to autumn: (1) massive emigration occurring immediately after release of the fish, (2) a period of little movement that spanned the summer months, and (3) an increase in downstream movement observed during early autumn. The spring and autumn movements appeared to be triggered by different factors. Emigrant fry during the immediate poststocking pulse of movement were smaller than the average size of resident fish (Fig. 2A−2D). This difference in size was consistent with the hypothesis that territorial interactions among recently emerged juvenile coho displace smaller, subordinate individuals (Chapman 1962; Mason and Chapman 1965). Significant differences (paired t-test, $p < 0.05$) between resident and emigrant fish were observed throughout the summer of 1982, the year of high initial population densities. There were no differences between the sizes of residents and emigrants during 1982 in the old-growth watershed. Lack of size differences between emigrant and resident coho during autumn suggested that territorial displacement was not the causative factor, but that a general emigration of the population was taking place.

Environmental Factors Triggering Emigration

The high rate of emigration immediately after stocking could not be attributed to any single environmental cue. Emigration always occurred following release of the fish, reached a peak 1−2 d later, and then declined precipitously afterwards (Fig. 1). Increases in downstream movement during the sum-

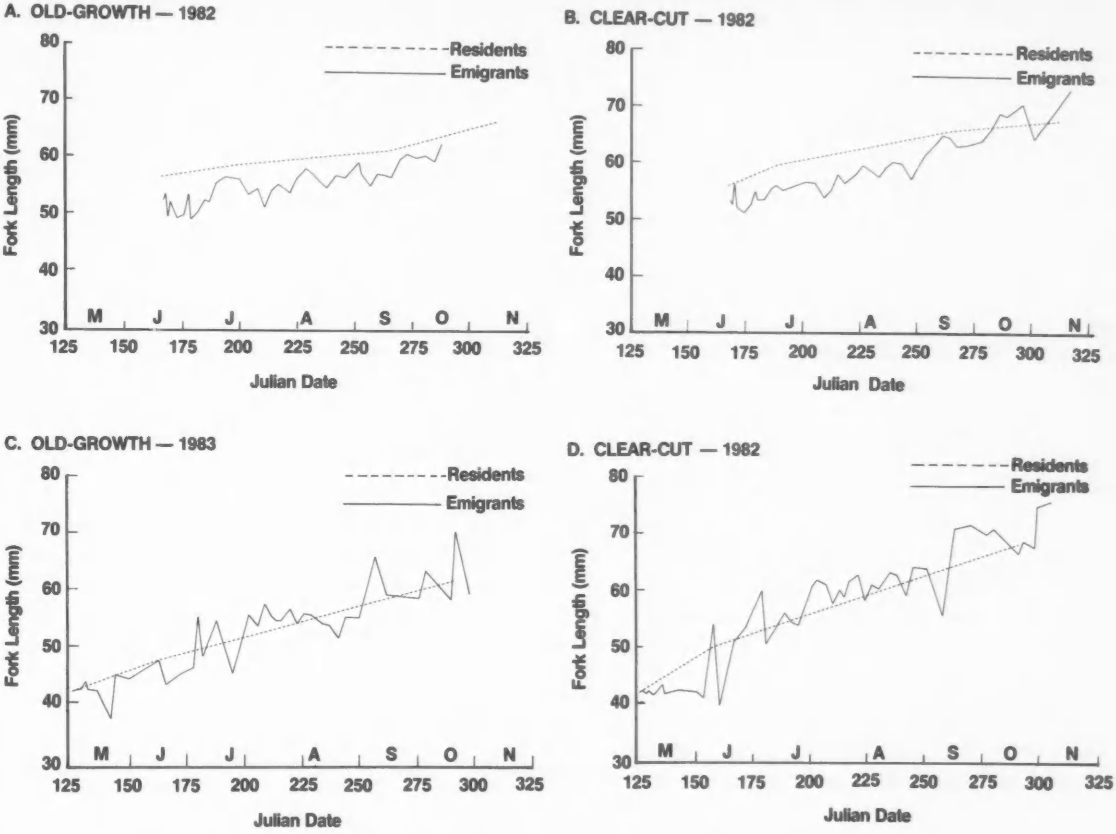

FIG. 2. Average lengths of emigrant and resident coho during (A and B) 1982 and (C and D) 1983.

mer and autumn coincided with flow and temperature changes. Streamflow in the old-growth stream was always higher than in the clear-cut (Fig. 3) owing to the larger watershed area. Substantially higher flows were experienced in 1983 than in 1982, when discharge was very stable throughout the summer. In 1982 peak flows were only 0.18 $m^3 \cdot s^{-1}$ in the clear-cut site and 0.36 $m^3 \cdot s^{-1}$ in the old-growth site. During the unusually wet summer of 1983, these values were exceeded repeatedly and peak mean daily flows were as great as 0.80 and 1.40 $m^3 \cdot s^{-1}$ in the clear-cut and old-growth watersheds, respectively. Freshets were often accompanied by increases in downstream movement of coho fry (Fig. 1A and 1B). This pattern was most evident in the clear-cut site, where increased emigration coincided with moderate flow increases during July and August. Flow changes in the old-growth stream during the summer of 1982 also appeared to coincide with periods of increased emigration, although the pattern was not as pronounced as it was in the clear-cut site due to the overall lower level of fish movement from this watershed. Periods of heavy emigration from both streams in the autumn also corresponded with increases in discharge.

The relationship between downstream movement and stream-flow differed somewhat between the two summers (Fig. 1C and 1D). In 1983, emigration increased with discharge up to a threshold level which was about 0.15 $m^3 \cdot s^{-1}$ in the clear-cut stream and 0.35 $m^3 \cdot s^{-1}$ in the old-growth site. Above these threshold discharges, downstream movement essentially ceased (Table 2). Flows that triggered the early autumn emigration of coho were, however, below these threshold values. Excluding the emigration rates immediately following stocking and those occurring when streamflow peaks exceeded the threshold, downstream movement from the streams in both years was primarily related to discharge changes (Table 3). There was an increase in the rate of movement whenever a change in daily flow >3% occurred. Flow changes greater than $3\% \cdot d^{-1}$ in early or midsummer elicited a mild increase in fry emigration, but discharge changes of a similar magnitude in early September resulted in much higher emigration rates.

Daily temperature maxima were generally higher and daily minima lower in the clear-cut study stream than in the old-growth site (Fig. 3C and 3D). Average diurnal temperature range during 1982 in the clear-cut stream was 4.7°C compared with 1.3°C in the old-growth stream. During the cooler, wetter summer of 1983, average diurnal temperature range in the clear-cut site was 3.4°C and in the old-growth site was 0.5°C. Midsummer temperature peaks in both streams were greater in 1982 than 1983. Differences in thermal regimes between the two streams were partly a consequence of riparian vegetation. The dense canopy over the old-growth site not only shaded the stream from direct sunlight during the day, but also acted as an

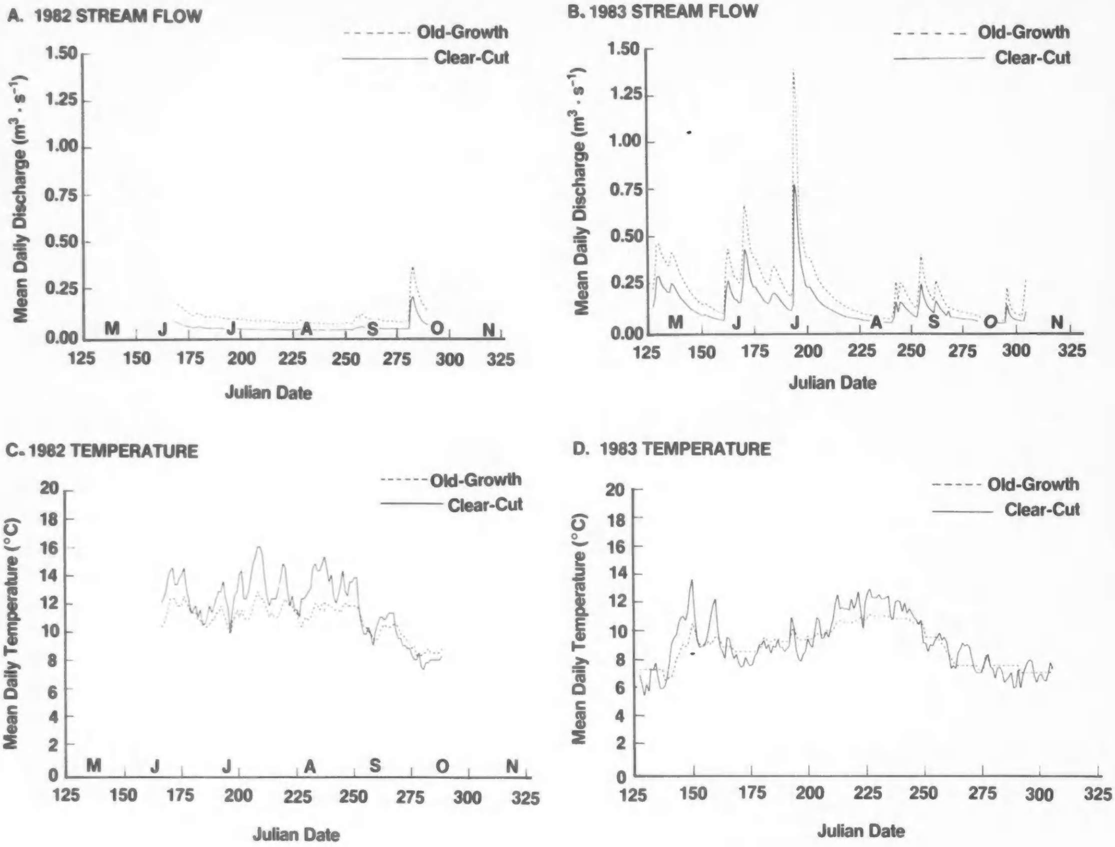

FIG. 3. Estimated (A and B) average daily streamflow and (C and D) average daily temperature during 1982 and 1983.

insulating layer at night, inhibiting heat exchange with the atmosphere and keeping water temperatures slightly warmer than in the clear-cut site. The larger water volume of the old-growth forested stream also contributed to its greater thermal stability.

TABLE 2. Average emigration rates during 1983 above and below threshold discharges of 0.15 $m^3 \cdot s^{-1}$ in the clear-cut and 0.35 $m^3 \cdot s^{-1}$ in the old-growth sites.

Site	Streamflow ($m^3 \cdot s^{-1}$)	Emigration rate (±1 SD) ($\% \cdot d^{-1}$)
Old-growth stream	≤ 0.35	0.12 ± 0.23
	>0.35	0.01 ± 0.03
Clear-cut stream	≤ 0.15	0.72 ± 1.75
	>0.15	0.06 ± 0.11

No clear association was found between changing water temperatures and emigration, although temperature decreases tended to produce higher rates of downstream movement than periods with stable or increasing mean daily temperatures (Table 3). In both watersheds, decreasing temperatures corresponded with emigration rates from 54 to 103% greater than those during periods of stable or rising temperatures; however, these differences were not significant. Although temperature changes alone did not result in significantly increased emigration, increases in the rates of movement during falling temperatures suggested that temperature may have acted together with flow change in triggering emigration. To determine the relative importance of flow change and decreasing temperature, average emigration rates were compared for periods of (1) changing flow and decreasing temperature, (2) decreasing temperature with no change in flow, (3) flow changes with no drop in temperature, and (4) no flow change or temperature decrease (Table 3). In both streams, decreasing temperature combined with daily flow changes >3% elicited the greatest emigration rates. In the old-growth site in 1982, these rates were greater than those observed during periods of either decreasing temperature alone or no flow and temperature change (t-test, $p \leq 0.10$). In the same site during 1983, decreasing temperatures combined with flow changes still produced the greatest average rate of downstream movement, but the average was not significantly different from those of the other flow and temperature conditions. Emigration from the clear-cut stream during changing flows and decreasing temperatures was greater than during periods of decreasing temperature alone or when there was no flow or temperature change (t-test, $p \leq 0.10$).

TABLE 3. Influence of flow change and temperature decreases on average daily emigration rates of coho after the initial pulse of emigration but excluding periods of peak discharge (Table 2).

Conditions	Coho emigration rate (±1 SD) ($\% \cdot d^{-1}$) Old-growth 1982	Old-growth 1983	Clear-cut 1982	Clear-cut 1983
Flow change $>3\% \cdot d^{-1}$	1.19 ± 0.39	0.18 ± 0.30	1.84 ± 1.79	1.12 ± 2.41
Flow change $\leq 3\% \cdot d^{-1}$	0.20 ± 0.15	0.05 ± 0.06	0.46 ± 0.27	0.22 ± 0.25
Decreasing mean daily temperature	0.61 ± 1.19	0.18 ± 0.38	1.11 ± 1.52	0.87 ± 2.39
Rising or stable mean daily temperature	0.39 ± 0.53	0.09 ± 0.11	0.70 ± 0.84	0.55 ± 0.61
Decreasing temperature and flow change $<3\% \cdot d^{-1}$	1.50 ± 2.02	0.27 ± 0.48	2.16 ± 2.45	1.41 ± 3.27
Decreasing temperature and flow change $<3\% \cdot d^{-1}$	0.21 ± 0.16	0.08 ± 0.04	0.56 ± 0.22	0.27 ± 0.25
Rising or stable temperature and flow change $\leq 3\% \cdot d^{-1}$	0.94 ± 0.80	0.09 ± 0.12	1.59 ± 1.30	0.69 ± 0.68
Rising or stable temperature and flow change $\leq 3\% \cdot d^{-1}$	0.19 ± 0.15	0.09 ± 0.08	0.38 ± 0.23	0.26 ± 0.23

TABLE 4. Emigration and mortality of hatchery coho during the initial pulse phase of emigration (first 10 d after stocking) and during the remaining summer and autumn study period. Mortality estimates assumed exponential death rates and were based on the difference between the known number of emigrants and residents.

	Clear-cut 1982	Clear-cut 1983	Old-growth 1982	Old-growth 1983
1. Coho stocked				
Total no.	13 090	7700	24 860	14 600
2. Emigration during first 10 d				
Total no.	5 376	5272	9 330	8 035
As % of no. stocked (1.)	41	68	38	55
3. Mortality during first 10 d				
Total	911	468	2 648	1 239
As % of no. stocked (1)	7	6	11	8
4. Resident population after 10 d				
Total no.	6 803	1960	12 882	5 326
5. Remaining emigration				
Total no.	2 091	255	1 617	316
As % of no. stocked (1)	16	3	7	2
As % of init. residents (4)	31	13	13	6
6. Remaining mortality				
Total no.	4 481	1421	11 153	3 823
As % of no. stocked (1)	34	18	45	26
As % of init. residents (4)	66	73	87	72
7. Population at end of study				
Total no.	231	284	112	1 187
As % of no. stocked (1)	2	4	<1	8
As % of init. residents (4)	3	14	1	22

Flow changes occurring without temperature declines resulted in the next highest emigration rates (Table 3), which were greater in both streams in 1982, and in the clear-cut site in 1983, than when temperature alone declined or when there were no flow or temperature changes (t-test, $p \leq 0.05$). In the old-growth site, downstream movements during periods of changing flow did not significantly differ from periods when either temperature decreased or there was no flow and temperature change in 1983. Declining temperatures alone resulted in emigration rates that differed little from those observed when neither temperature nor flow changed. Discharge, therefore, appeared to be the physical factor of overriding importance in triggering the downstream movement of hatchery coho fry following the initial pulse of poststocking emigration. Our results showed that temperature influenced downstream movement only when accompanied by flow changes.

Average daily turbidities in 1983 were 1.3 NTU in the old-growth and 1.2 NTU in the clear-cut site with peak values of 5.7 NTU and 5.5 NTU, respectively. These peak values were well below levels known to elicit an avoidance response by coho salmon fry (Berg 1982; Bisson and Bilby 1982; Berg and Northcote 1985), and no relationship between turbidity and downstream movement was observed. Due to the consistently clear water in both streams, it was not possible for us to draw any meaningful conclusions about the influence of water clarity on coho fry emigration. Like Hartman et al. (1982), we also saw no influence of lunar phase on downstream movement of coho fry (Fig. 1A and 1B); however, Mason (1975) found coho fry emigration to increase around the new moon.

Survival, Growth, and Production

The initial emigration rate was greater in 1983 than 1982, despite lower stocking densities (Table 4). In 1983, coho were planted 1 mo earlier and at a much smaller size than those planted in 1982, and the streams experienced marked flow increases in 1983. These factors may have contributed to the relatively high 1983 initial emigration rate. Once the poststocking pulse of emigration subsided, however, downstream movement from both streams remained consistently lower in 1983 than in 1982 when stocking densities were high (Fig. 1; Table 4). During both years, a greater percentage of stocked fish initially moved out of the clear-cut site than left the old-growth site. After the populations had stabilized, emigration from the clear-cut was approximately twice the rate of emigration from the old-growth stream.

Initially, mortality differed little between the study sites, although mortality estimates for the old-growth were slightly greater than those for the clear-cut site in both years (Table 4). During the early period of population decline, emigration accounted for far more fish than mortality. Following population stabilization, mortality became the dominant process and accounted for the majority of coho that disappeared from the streams. In 1982, when initial resident population densities were high, mortality in the old-growth stream was about 20% greater than in the clear-cut stream. At the low population densities of 1983, mortality rates for the two sites were identical. When the initial poststocking phase of movement and the remaining summer period of relative population stability were considered together, emigration generally proved to be more important than mortality in regulating population sizes. This was especially true for the clear-cut site, where emigration accounted for 57 and 79% of the number of coho stocked in 1982 and 1983, respectively. In the old-growth stream, emigration accounted for 57% of the stocked fish in 1983, but only 44% in 1982 when initial densities were high. In both years, only a small number of coho remained at the end of the monitoring period (Table 4), but it was revealing that in each site, more fish remained in the streams during the year when stocking densities were low than when initial densities were high, the most marked difference being in the old-growth site where 10 times as many coho remained at the end of the study during the low stocking density year than during the high-density year.

A possible reason for the increased tendency of coho fry to reside in the old-growth stream was that more suitable habitat was available there than in the clear-cut site. The old-growth stream had a much higher frequency of woody debris than the clear-cut, which had been cleared of nearly all woody debris following logging. Large woody debris is instrumental in the formation of pools in small streams (Swanson et al. 1976), and pool habitat is preferred by coho salmon fry (Hartman 1965; Ruggles 1966; Lister and Genoe 1970; Nickelson and Hafele 1978). Woody debris serves as a source of cover for the fish (Bustard and Narver 1975a, 1975b; Bisson et al. 1982; Forward 1984) and by visually isolating the fry from one another, may reduce aggressive interactions and enable more fish to establish foraging sites in a smaller area of stream than would be possible otherwise (Dolloff 1983). The greater availability of suitable habitat in the old-growth stream may have initially slowed emigration, which in turn led to intense competition for available food and the observed higher mortality rates, particularly in the high-density year. Rapid emigration of coho fry from the clear-cut site may have been a result of lack of pool habitat and cover, which led to competition for the available space.

Production in the two streams reflected differences in the apparent availability of both food and usable rearing space, and the compensatory controls these two resources exerted over the coho populations. In the high-density year (1982), production through mid-September was severalfold greater in the clear-cut site than in the old-growth site (Table 5), largely due to differences in growth rates. In the old-growth site, daily growth rates increased during the autumn. By this time the population had declined to a small fraction of the number of fish originally stocked (Table 4) and the impact of increased growth on production was very small. Total coho salmon production during the 1982 study period was 5.28 $g \cdot m^{-2}$ in the clear-cut stream and 2.05 $g \cdot m^{-2}$ in the old-growth stream; however, this difference in production was not reflected in mean individual weights, which were nearly equal in the two streams at the end of the study.

In 1983, the low-density year, growth during the first month in both streams was greater than during the first month's residence in 1982 (Table 5), but fish in the clear-cut site still grew at a faster rate than those in the old-growth site, although the difference between streams was less than in 1982. Growth rates remained higher than comparable intervals in 1982 throughout the balance of summer and early autumn in both streams. At the end of the 1983 study, coho in the clear-cut site were about 30% larger than those in the old-growth site, based on superior early-season growth. Total production from May to mid-October was 4.63 $g \cdot m^{-2}$ in the clear-cut stream and 3.95 $g \cdot m^{-2}$ in the old-growth stream. Thus, when high densities were stocked in the study streams, production in the clear-cut site was clearly greater than in the old-growth site. At lower stocking densities the clear-cut site still maintained higher production rates than the old-growth stream, but differences between sites were diminished.

The results indicate that the rate of emigration of hatchery coho from the study streams was strongly influenced by habitat quality whereas growth (and consequently production) was primarily related to food availability and population density. The old-growth stream possessed high-quality habitat containing an abundance of pools and cover, and the number of coho remaining at this site after the initial population had stabilized was greater than at the clear-cut site. Growth rates, however, were lower and mortality rates greater in the old-growth site than in the clear-cut site, which suggested that scarce food was

TABLE 5. Growth, biomass, and production of coho in the clear-cut and old-growth streams during 1982 and 1983. Yearly averages are weighted according to length of sampling interval.

Stream	Sampling interval	Avg. wt. per fish at end of interval (g)	Daily instantaneous growth rate	Average population biomass ($g \cdot m^{-2}$)	Production ($g \cdot m^{-2} \cdot d^{-1}$)
1982					
Clear-cut	June 15 – July 7	2.11	0.0052	20.59	0.108
Old-growth	June 15 – July 18	1.94	0.0010	19.72	0.020
Clear-cut	July 7 – Sept. 21	2.80	0.0037	9.16	0.034
Old-growth	July 18 – Sept. 22	2.20	0.0019	6.09	0.012
Clear-cut	Sept. 21 – Nov. 8	3.10	0.0021	3.01	0.006
Old-growth	Sept. 22 – Nov. 9	3.00	0.0065	2.04	0.013
Clear-cut	1982 weighted averages		0.0034		0.036
Old-growth			0.0032		0.014
1983					
Clear-cut	May 6 – June 7	1.56	0.0249	3.71	0.092
Old-growth	May 6 – June 13	1.25	0.0146	3.63	0.053
Clear-cut	June 7 – Oct. 18	4.25	0.0075	1.77	0.013
Old-growth	June 13 – Oct. 19	3.23	0.0074	2.03	0.015
Clear-cut	1983 weighted averages		0.0109		0.028
Old-growth			0.0090		0.024

TABLE 6. Gross primary production and average light intensities in the two study streams during sunny and cloudy weather in 1983.

Stream	Weather conditions	Mean light intensity during incubation (±1 SD) ($g\ cal \cdot cm^{-2} \cdot min^{-1}$)	Carbon fixation (±1 SD) ($mg\ C \cdot m^{-2} \cdot h^{-1}$)
Old-growth	Sunny	0.32 ± 0.19	26.8 ± 7.6
Old-growth	Cloudy	0.06 ± 0.02	16.6 ± 6.0
Clear-cut	Sunny	1.18 ± 0.08	51.7 ± 12.3
Clear-cut	Cloudy	0.32 ± 0.01	29.5 ± 5.9

being apportioned among more individuals. While food limitation of coho production appeared to be particularly severe in the old-growth watershed, evidence of limiting food resources also existed in the clear-cut stream. In both sites, growth rates were lower at high stocking densities than at low stocking densities (Table 5), and competition for foraging stations may have resulted in the downstream displacement of smaller fish (Fig. 2). Similar size differences between resident and emigrant wild coho fry have been observed in Carnation Creek (Hartman et al. 1982).

Our study streams were probably overstocked with coho fry in 1982; fewer fish remained at the end of the 1982 monitoring period, and their average weights were less than in the following year when only about half as many fish were stocked. In 1983, production of coho in the clear-cut site was similar to the production measured in 1982, yet the initial resident population in 1983 (Table 4) was approximately one third of the number of fish in 1982. Production of coho in the old-growth stream in 1983 was nearly double the total 1982 production, but with less than one half the initial number of residents. Because there was virtually no change in habitat composition or cover in the two streams from 1982 to 1983, we infer that production differences between years were primarily a function of limited food availability coupled with changes in population density.

Although we did not measure food abundance directly, there was additional evidence for increased food resources in the clear-cut watershed. On both sunny and cloudy days, gross primary production in the clear-cut stream was approximately twice that of the old-growth stream (Table 6). From frequent visual observations, it was apparent that the clear-cut stream contained a periphyton assemblage dominated by filamentous green algae that supported numerous grazing insects whereas the periphyton of the old-growth was dominated by epilithic diatoms. It is also possible that algal growth in the clear-cut stream may have been enhanced by slightly increased temperatures and elevated nutrient export from the watershed after logging. The primary production measurements and the observed differences in coho growth between the two streams both support the hypothesis that food was more abundant in the clear-cut site than in the old-growth site during the study period. Our finding that habitat quality exerts a significant influence on the frequency of downstream movement of hatchery coho fry, and hence local population density, while food resources tend to control individual growth rates, and hence production of the population, is in general agreement with results of the Carnation Creek study of wild coho. In Carnation Creek, Scrivener and Andersen (1984) observed that underyearling coho growth in different reaches was negatively correlated with density during summer, even when the relative abundance of fish was low, implying that intraspecific competition for food was taking place regardless of the size of the initial population or the quality of physical habitat. These authors also found that differences in growth between years were density related and that removal of streamside vegetation during logging resulted in summer growth increases that yielded larger fry in early autumn, except in years when adult escapement and subsequent initial fry populations had been unusually high. The larger fry in early autumn were felt to have a greater probability of surviving until smoltification. Tschaplinski and Hartman (1983) showed that more coho fry remained in stream reaches with deep pools, log jams, and undercut banks, which provided suitable overwintering habitat, than in reaches where pools and cover were lacking; in the latter sites the rate of downstream movement of fry in response to freshets was very high. Although their research was conducted in autumn and winter (the period when our study was ending), the migratory behavior of wild coho fry in response to habitat quality in Carnation Creek appeared to be quite similar in many respects to the behavior we observed in hatchery fish.

The success of hatchery fry outplanting programs, as measured in terms of increased standing stock or smolt yield, is often based on the concept that to realize its production potential a stream must be "fully seeded." We suggest that this concept will require careful definition as well as clarification of management objectives. If full seeding is taken to mean the maximum *number* of fish that can live in a given set of physical conditions in the stream channel, our study indicated that high densities may result in poor growth, possibly at the expense of survival to smolting. We found that the number of fish remaining in the study reaches after stocking appeared to be more a function of habitat quality than of trophic conditions. Where a stream contains abundant pools and cover, we predict that more coho fry will remain in the vicinity of where they are planted than in a stream where these features are scarce, regardless of the availability of food. In streams possessing high quality habitat, it may therefore be desirable to scatter the planting sites along the entire length of the channel in order to ensure an even distribution of fry throughout the drainage. Conversely, single location plants in streams with poor physical habitat can be expected to result in considerable downstream movement, resulting in occupation of suitable rearing areas elsewhere in the system. If full seeding is taken to mean the density that yields maximum *production*, our results showed that greater coho fry production could be achieved, at least in the old-growth site, at a lower density than the maximum number of fish the stream could support. Overstocking a stream in which food resources are severely limited can result in lower growth rates and higher mortality. We conclude that fry planting programs should taken into account the relative availability of food in a stream, not just physical habitat quality, and adjust stocking densities accordingly.

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Depression of Primary Production by Humic Matter and Suspended Sediment in Limnocorral Experiments at Southern Indian Lake, Northern Manitoba

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Guildford, S. J., F. P. Healey, and R. E. Hecky. 1987. Depression of primary production by humic matter and suspended sediment in limnocorral experiments at Southern Indian Lake, northern Manitoba. Can. J. Fish. Aquat. Sci. 44: 1408–1417.

Eroding and flooded shoreline materials were added to a series of limnocorrals in Southern Indian Lake (northern Manitoba) to simulate their effects on the phytoplankton following impoundment of the lake. Inorganic clays depressed primary productivity and phytoplankton biomass but relieved phosphorus deficiency. These effects appeared to be primarily due to reduction of light penetration; release of available phosphorus was small. Organic moss–peat material initially increased and then lowered primary productivity and biomass, while phosphorus deficiency was lowered. The initial stimulation could be traced to the release of soluble nitrogen and phosphorus. Enrichment experiments indicated that the depression was due to the binding of iron or some other metal by dissolved humic material. Responses of phytoplankton in the lake could be understood as a composite of responses seen in the limnocorrals to the two eroding shoreline materials.

Des matériaux recueillis de rives érodées et inondées ont été ajoutés à une série d'enclos dans le lac Southern Indian (nord du Manitoba) afin de simuler leur incidence sur le phytoplancton après l'endiguement du lac. Les argiles inorganiques ont abaissé la productivité primaire et la biomasse phytoplanctonique mais ont allégé la carence en phosphore. Ces incidences semblent être surtout le résultat d'une diminution de la pénétration de la lumière; la libération de phosphore disponible était faible. La présence de sphaigne organique a entraîné une augmentation initiale puis une diminution de la productivité primaire et de la biomasse tandis que la carence en phosphore a été réduite. La stimulation initiale peut être retracée au lâcher d'azote et de phosphore solubles. Des expériences d'enrichissement révèlent que cet abaissement est le résultat de la fixation du fer ou d'un autre métal par les matières humiques dissoutes. Les réactions du phytoplancton lacustre peuvent être considérées comme une composante des réactions du phytoplancton, en présence des deux types de matériaux provenant de rives érodées, observées dans les enclos.

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In 1976, the water level of Southern Indian Lake (SIL) was raised 3 m to divert the Churchill River southward to hydroelectric generating stations located on the Nelson River. An intensive multidisciplinary study of the lake–reservoir before, during, and after impoundment and diversion was conducted at SIL during the years 1973–78 (see Can. J. Fish. Aquat. Sci. 41: 547–734). From 1981 to 1982, experiments using large enclosures were conducted at SIL to investigate some of the hypotheses formed to explain changes in the lake observed after impoundment.

After impoundment, mean integral primary productivity decreased in areas of the lake where the daily mean water column light intensity (I_{24}) fell markedly due to increased suspended sediments. In other areas of the lake where there was little change in I_{24}, primary productivity remained constant or increased to the extent that mean integral primary productivity for the lake as a whole did not change from pre- to post-impoundment. Prior to impoundment, all areas of SIL were moderately to severely phosphorus deficient as demonstrated by indicators of algal nutrient status such as alkaline phosphatase activity and nutrient composition. After impoundment, indications of P deficiency decreased or disappeared completely (Hecky and Guildford 1984). In the first 3 yr after flooding, it was estimated that 4×10^6 t of mineral sediment and 6×10^4 t of organic material (G. K. McCullough, Freshwater Institute, pers. comm.) were eroded annually to SIL. This was a 20-fold increase in sedimentary input to the lake (Hecky and McCullough 1984). Thus, results were explained by hypothesizing that in areas where I_{24} became extremely low due to increased suspended sediments and increased mixing depths, integral productivity declined because algae became light limited. In other areas of the lake where the reduction of I_{24} was less, integral primary productivity increased or remained constant because of increased P loading from eroding shoreline materials.

Two types of shoreline material were added to the limnocorrals designed to test hypotheses concerning algal nutrient status and primary productivity: inorganic fine-grained, glaciolacustrine clay material, which was the predominant overburden in the area of SIL, and the organic moss layer with underlying peat which formed the main ground cover for the area.

The specific questions addressed were as follows: (1) Were

TABLE 1. Limnocorral code names, treatments, bottom type, mean depth, and seasonal means of Secchi disk depth, light extinction coefficient (k), and total suspended sediment (TSS). ND = not done.

Name	Treatment	Bottom type	Depth (m)	Secchi (m)	k (m^{-1})	TSS ($mg \cdot L^{-1}$)
	Sediment testing					
LS81-4	Low sediment; sediment added biweekly to obtain Secchi depth of 0.50 m (total 32 kg dry wt)	Plastic	4.02	0.8	1.7	10.1
HS81-6	High sediment; sediment added biweekly to obtain Secchi depth of 0.30 m (total 52 kg dry wt)	Plastic	4.01	0.5	2.2	14.6
HS82-7	High sediment; sediment added weekly to obtain Secchi depth of 0.35 m (total 52 kg dry wt)	Natural	2.90	0.5	2.0	ND
SC82-2	Screen; surface completely covered with one thickness of black fibreglass screen	Natural	2.70	2.3	1.1	ND
	Moss−peat testing					
LM81-2	Low moss; 63 kg dry wt moss−peat added June 21, 1981	Natural	3.50	2.3	1.1	2.4
LM82-3	Low moss; 52 kg dry wt moss−peat added June 28, 1982	Natural	3.60	2.4	1.1	ND
HM82-4	High moss; 104 kg dry wt moss−peat added June 28, 1982	Natural	2.70	2.0	1.3	ND
OM82-8	Old moss; moss−peat limnocorral studied for 2 yr (previously LM81-2)	Natural	3.20	2.4	1.0	ND
	Controls					
C81-1	Control	Natural	2.81	1.8	1.0	3.6
C81-5	Control	Plastic	3.48	2.4	0.8	2.5
C81-7	Control	Plastic	3.48	2.4	0.8	2.2
OC82-5	Old control; used for 2 yr (previously C81-1)	Natural	3.30	2.3	1.0	ND
C82-6	Control	Natural	2.90	2.1	1.1	ND
	Lake					
L81-8	Lake			1.5	1.1	4.0
L82-9	Lake			1.3	1.2	ND

eroding clays a significant source of available P which contributed to the overall decrease in P deficiency observed in SIL after impoundment, or did P deficiency decrease because light limitation due to increased suspended materials reduced P demand by algae? (2) Was inundated moss−peat material a significant source of available P which would stimulate primary productivity, or would humic matter leached from the moss−peat material depress primary productivity as observed by Sakamoto (1971), Jackson and Hecky (1980), and Arvola (1984)? To examine this latter aspect, a series of bottle enrichment experiments were conducted to test for metal limitation of phytoplankton growth in the moss treatment limnocorrals.

Methods

All limnocorrals were 10 m in diameter and made of reinforced translucent polyethylene (Guildford 1985). Four had plastic bottoms; the remaining nine were open to the bottom sediments. The limnocorrals are identified by letters to indicate treatment and numbers to indicate year and limnocorral number (Table 1). For example, the low-sediment treatment was limnocorral number four in 1981, that is LS81-4, and the 1-yr-old control studied in 1982 was limnocorral number five, that is OC82-5 (Table 1).

Limnocorral Treatments

SIL shoreline glacio-lacustrine clay (Erosion site 4 of Newbury and McCullough 1984) was added to three limnocorrals (LS81-4, HS81-6, HS82-7) throughout two summer seasons. The clay was brought into suspension in a large plastic container, added to the limnocorral as a slurry, and dispersed with an electric outboard motor. Sufficient sediment was added weekly or biweekly to maintain Secchi disk values comparable with moderately and extremely turbid areas in the lake. A fourth limnocorral (SC82-2) had no sediment added but was covered with a screen to reduce light penetration to the same degree as one of the sediment treatments. This was done to observe the effect of reduced light alone, without the influence of the sediments.

Moss and peat material, taken from a shoreline of Wupaw Bay, an arm of SIL (Newbury et al. 1984), was added to three limnocorrals (LM81-2, LM82-3, HM82-4). The material was added all at one time to simulate flooding. The proportion of flooded moss to water volume in the low-moss limnocorrals was designed to be the same as that in Notigi reservoir which was flooded in 1973 (Newbury et al. 1984). The effect of moss−peat after 1 yr under water was observed by monitoring a moss−peat limnocorral (OM82-8) 2 yr in a row. At the beginning of the second summer the sides of this limnocorral were lowered for 2 d to allow circulation of lake water.

Four untreated limnocorrals (C81-1, C81-5, C81-7, C82-6) were studied as controls. One of these (C81-1) was studied 2 yr in succession. In 1982 it was called OC82-5. Each year, samples from the lake just outside the limnocorrals were studied as well.

Field and Laboratory Methods

Samples were collected biweekly in 1981 and weekly in 1982 using an integrated sampler (Fee 1976) which sampled the euphotic zone (1% of surface light) or down to 2.5 m, whichever was less. Single integral samples were considered representative of the limnocorrals because they did not stratify thermally. Irradiance, Secchi disk depth, and temperature were measured at the time of sample collection according to Hecky (1984). Irradiance data were used to calculate the vertical light extinction coefficient k (Hutchinson 1957) and the mean water column light intensity (Fee 1979; Ramberg 1979; Hecky and Guildford 1984; Guildford 1985). The mean water column light intensity over 24 h, I_{24}, was calculated using the following equation:

$$I_{24} = \frac{I_0\,(1 - e^{-kz})}{kz}$$

where I_0 is mean surface irradiance over 24 h (cloudless irradiance data (Fee 1977) was used to facilitate comparisons between days and years), k is the vertical light extinction coefficient, and z is the depth of the limnocorral.

Water samples were analysed for chlorophyll *a*, particulate carbon, particulate nitrogen, particulate phosphorus, total suspended sediment (TSS), dissolved inorganic carbon (DIC), total dissolved nitrogen (TDN), total dissolved phosphorus (TDP), and soluble reactive phosphorus (SRP) by methods given in Stainton et al. (1977).

Humic content was estimated by measuring the absorbance at 455 nm (A_{455}) of lake water following filtration (Whatman GF/C) and centrifugation (13 000 × g for 20 min).

ATP was extracted from water samples and measured using the method of Rudd and Hamilton (1973). ATP measurements from the sediment limnocorrals were not used because high concentrations of suspended sediments interfered with the extraction, causing up to 90% underestimation of ATP (Guildford 1985).

Rates of primary productivity were estimated using a ^{14}C incubator technique similar to that described by Shearer and Fee (1974). The irradiance at which photosynthesis begins to be saturated, I_k, was calculated from the incubator-generated light response curves (Hecky and Guildford 1984). Daily integral production was calculated using the computer program designed by Fee (1973) which combines the light response in the incubator, light extinction in situ, and cloudless irradiance.

Alkaline phosphatase activity (Healey and Hendzel 1979a) was used as an indicator of P deficiency. Rates were normalized to ATP except in the sediment limnocorrals where chlorophyll was used.

P debt and N debt were used as indicators of P and N deficiency, respectively (Healey and Hendzel 1979b). Particulate nutrient composition ratios (N/C, P/C, N/P) were also examined as indicators of nutrient status (see Table 3).

Enrichment Experiments

During the first year of limnocorral experiments, it became apparent that rates of primary productivity and chlorophyll concentrations in the moss−peat treatment limnocorral (LM81-2) were lower than in the control (C81-1). This suggested that humic material leached from the moss−peat material may have been binding metals normally in solution causing the algae in the low-moss limnocorral to be metal limited. To test this hypothesis, three enrichment experiments were conducted on water from the low-moss limnocorrals and their corresponding control limnocorrals in both 1981 and 1982 (Table 2). The first two experiments were done in flasks in the laboratory and the third experiment was done in 20-L containers in situ. Variables monitored in all three experiments were chlorophyll *a*, photosynthesis, and alkaline phosphatase activity. In the second enrichment experiment, moss−peat material dredged from the bottom of the low-moss limnocorral (LM82-3) was added to water from the low-moss (LM82-3) and control limnocorrals (C82-6). This was done to determine whether it was necessary for the moss−peat material to be present for the growth-inhibiting effect observed in the low-moss limnocorrals to continue or whether the algal growth-inhibiting effect was present in dissolved material leached from the moss−peat material. The amount of moss−peat material added to the flasks was in the same proportion to flask volume as that added to the low-moss limnocorral (LM82-3) at the beginning of the summer. As well as testing the hypothesis that algae in the low-moss limnocorral were Fe limited, the third experiment was also designed to test whether the algae might be light limited due to leached humic matter reducing transparency and/or altering light quality. To test for light limitation, replicate containers were suspended at two depths, 0 and 0.8 m, in the low-moss limnocorral. An additional unenriched container was covered with a screen and suspended at the surface of the low-moss limnocorral. The light intensity inside this container was the same as that in the untreated container suspended at 0.8 m. If light was limiting in the low-moss limnocorral, then comparison of these two containers would reveal whether changes in light quality, due to selective absorption of light by humic matter in the water column, might be responsible for limitation of primary productivity.

TABLE 2. Details of the three bottle enrichment experiments conducted on water from the low-moss limnocorrals (LM81-1, LM82-3) and their control limnocorrals (C81-1, C82-6).

Limnocorrals sampled	Bottle volume (L)	Treatments	Light ($\mu E \cdot m^{-2} \cdot s^{-1}$)	Days subsampled
		Enrichment experiment I (August 4–11, 1981)		
Low moss (LM81-2)	0.5	1. No enrichment	54	4, 7
Control (C81-1)		2. $FeCl_3 \cdot 6H_2O$ + Na_2EDTA[a]		
		3. Na_2EDTA[b]		
		4. Trace metals[c]		
		Enrichment experiment II (August 1–7, 1982)		
Low moss (LM82-3)	3	1. No enrichment	200	1, 3, 4, 6
Control (C82-6)		2. $FeCl_3 \cdot 6H_2O$ + Na_2EDTA[a]		
		3. Moss[d]		
		Enrichment experiment III (August 1–10, 1982)		
Low moss (LM82-3)	20	1. No enrichment, 0 m	In situ	0, 2, 5, 9
		2. No enrichment, 0.8 m		
		3. $FeCl_3 \cdot 6H_2O$ + Na_2EDTA[a], 0 m		
		4. $FeCl_3 \cdot 6H_2O$ + Na_2EDTA[a], 0.8 m		
		5. Moss, 0 m[d]		
		6. Moss, 0.8 m[d]		
		7. Screen cover, 0 m		

[a] Final concentration 2.34 μM $FeCl_3 \cdot 6H_2O$ and 2.34 μM Na_2EDTA.

[b] Final concentration 2.34 μM Na_2EDTA.

[c] Final concentrations are 1/5 those used in medium WC (Guillard and Lorenzen 1972).

[d] 2.5 g wet wt·L $sample^{-1}$.

Results and Discussion

Sediment Limnocorrals

The type of sediment added to the limnocorrals was a glaciolacustrine, silty clay, mainly inorganic (total C/N/P ratio 23.0:0.6:0.6 mg·g dry wt^{-1}, 70% of total C was carbonate), very fine grained (85% clay; Newbury and McCullough 1984), and greyish in colour (Guildford 1985). About 2% of total sediment P was extractable in distilled water as SRP (Guildford 1985).

A substantial fraction of sediment added to the limnocorrals remained in suspension rather than settling out, due to the fine grain size. The concentration of TSS and the light extinction coefficient (k) were higher in all the sediment limnocorrals than in the control (Table 1). As a result, I_{24}, which is a function of k and depth, was significantly less in the sediment limnocorrals (Table 3).

Integral primary production in the sediment-treated and screen-covered limnocorrals was 25–50% lower than in the controls and lake (Table 3). Biomass, as estimated by chlorophyll and suspended C, was similar to or greater than the controls and lake in the screen-covered and all sediment limnocorrals except HS81-6 (Table 3). The nutrient status indicators (P debt, alkaline phosphatase activity, and N/P and P/C rations) (Table 3) showed that there was less P deficiency in the sediment limnocorrals than in the control or the screen-covered limnocorrals. None of the limnocorrals was N deficient (Guildford 1985).

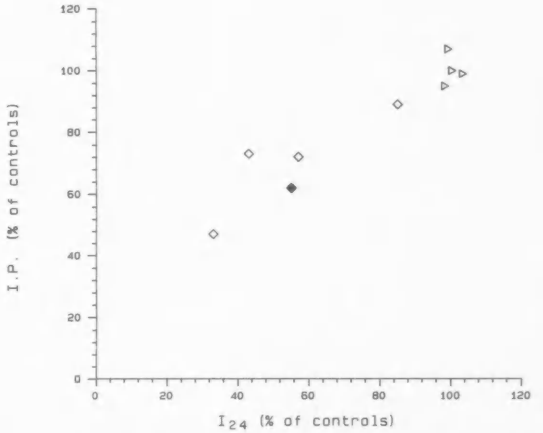

FIG. 1. Relationship between daily integral primary productivity (I.P.) and mean daily water column light intensity (I_{24}) in the sediment (◇), screen-covered (◆), and control (▷) limnocorrals from both 1981 and 1982. The data are time-weighted means expressed as a percent of the appropriate controls in order to intercompare the different years. Individual control limnocorral values are expressed as a percent of the mean of controls for each year in order to show the range of variability between controls.

The principal effect of sediment addition was a reduction in primary productivity. This appeared to be due to light reduction rather than any physiological interaction between algae and sediments. The similar decreases in primary productivity in both the sediment and screen-covered limnocorrals and the correlation between primary productivity and I_{24} (Fig. 1, r = 0.95) support this point. Light limitation in the sediment and screen-covered limnocorrals is also indicated by the higher

TABLE 3. Seasonal means of sediment limnocorral data. I.P. = integral primary productivity; I_{24} = mean water column light intensity; I_k = irradiance at the onset of light saturation; Pase = alkaline phosphate activity (μmol *o*-methylfluoroescein phosphate hydrolyzed$\cdot h^{-1}\cdot$μg chlorophyll^{-1}); Pdebt = estimated μmol phosphate removed in 24 h of darkness$\cdot$μg chlorophyll^{-1}; Susp. C = suspended carbon. Nutrient ratios are particulate. ND = not done.

Limnocorral	Treatment	I.P. (mg $C\cdot m^{-2}\cdot d^{-1}$)	I_{24} ($E\cdot m^{-2}\cdot d^{-1}$)	I_k ($E\cdot m^{-2}\cdot d^{-1}$)	Pase[a]	Pdebt[b]	P/C[c] (μg$\cdot$mg^{-1})	N/P[d] (μg$\cdot$μg^{-1})	Chl *a* (μg$\cdot L^{-1}$)	Susp. C (μg$\cdot L^{-1}$)
1981										
C81-1	Control	500	21.1	17.1	0.146	0.285	14	16	3.9	719
C81-5	Control	480	20.0	16.4	0.155	0.422	9	21	3.6	787
C81-7	Control	541	20.4	17.0	0.173	0.476	11	17	3.0	701
LS81-4	Sediment (32.6 kg)	369	8.7	15.6	0.124	0.300	16	11	4.6	813
HS81-6	Sediment (52.4 kg)	239	6.7	16.0	0.077	0.192	32	6	2.8	474
L81-8	Lake	449	17.4	19.3	0.056	0.204	20	9	2.5	435
1982										
C82-6	Control	290	18.7	19.3	0.026	ND	11	20	2.2	909
HS82-7	Sediment (51.5 kg)	208	10.6	20.6	0.003	ND	21	10	3.2	1019
SC82-2	Screen	181	10.2	17.3	0.019	ND	12	17	2.7	909
L82-9	Lake	ND	ND	ND	0.008	ND	15	15	2.1	644

[a] 0.003–0.005 = moderate, >0.005 = severe P deficiency.

[b] >0.075 = P deficiency.

[c] 10–20 = moderate, <10 = severe P deficiency.

[d] >10 = P deficiency.

chlorophyll/C ratios and the low ratios of I_{24}/I_k. Elevated chlorophyll/C ratios are a common response to light limitation (Healey 1985), while values of I_{24}/I_k below 1 suggest light limitation (Hecky and Guildford 1984).

The greater degree of P deficiency in the screen-covered limnocorral (SC82-2) relative to the sediment limnocorral (HS82-7), as reflected by the higher phosphatase activity and lower P/C ratio of the former (Table 3), shows that some P was released by the sediments; but the marginal difference in integral productivity and biomass between these two limnocorrals (Table 3) shows that this release was small. This is not surprising, since in general sediments that have a high proportion of available P are those associated with agricultural watersheds (Dorich et al. 1980; McColl 1975) or wastewater (Young and DePinto 1982). The SIL shoreline sediments were similar to the Lake Erie Bluff sediments described by Williams et al. (1980) and Young and DePinto (1982). Both sediments were mainly inorganic with about 0.6 mg P$\cdot$g dry wt^{-1}, and less than 5% of total P was extractable using mild extraction techniques (Williams et al. 1980; Guildford 1985). In algal bioassay experiments (Williams et al. 1980) the inorganic Lake Erie Bluff sediments would not support the growth of P-starved algae.

Some degree of P deficiency was still detectable in two of the three sediment limnocorrals and in the screen-covered limnocorral. Since all these limnocorrals were also light limited, it appears that two factors, light and P, were limiting these populations simultaneously. In laboratory cultures a single species can be both light and nutrient limited over a narrow range of light intensities and nutrient concentrations because to a degree, a physical factor can compensate for a nutrient and vice versa (Rhee and Gotham 1981; Healey 1985). In the limnocorrals, this apparent simultaneous limitation may also have been due to different species being limited by different factors (Titman 1976).

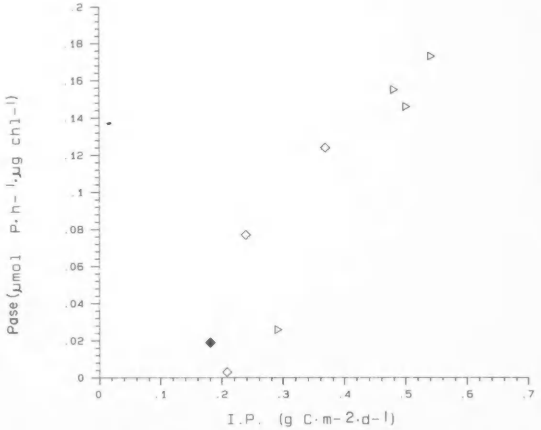

FIG. 2. Relationship between daily integral primary productivity (I.P.) and alkaline phosphatase activity (Pase) in the sediment (◇), screen-covered (◆), and control (▷) limnocorrals from both 1981 and 1982. The data are time-weighted mean values for each year.

Because the sediment and screen-covered limnocorrals had lower rates of integral productivity than the controls, it is surprising that they did not have similarly lower concentrations of biomass as estimated by suspended C and chlorophyll. Only HS81-6 (the most severely light-limited limnocorral) had lower biomass than the controls. This discrepency between productivity and biomass may have been due to the lower zooplankton populations in the sediment and screen-covered limnocorrals (Guildford 1985).

Estimates of P loading into new reservoirs as a result of flooding are often made to predict the changes in productivity of the new reservoirs (Ostrofsky 1978). The sediment limnocorral experiments showed that, although increased suspended sediment resulted in a decrease in P deficiency (and probably released a small amount of P), the decrease in P deficiency was mainly due to the decrease in integral primary productivity (Fig. 2) rather than an increase in available P. In the sediment limnocorrals the decreased light availability relieved the dependence of algal productivity on P supply. Similarly in new reservoirs with reduced light penetration and/or greater mixed depth, algal productivity may not be dependent on P loading.

Moss–Peat Limnocorrals

The material added to the moss–peat limnocorrals was predominantly peat with overlying sphagnum and feather mosses. The material was mainly organic (total C/N/P ratio 380.0:4.0:0.4 mg·dry wt^{-1}) with 2% of total C being carbonate.

The main physical change observed after the moss–peat addition was in water colour. Absorbance at 455 nm, used here as an estimator of dissolved humic material (DHM), was consistently higher in the moss–peat limnocorrals than in the controls (Table 4). The concentrations of TDP and TDN also rose after the addition of moss–peat material (Table 4). Increased concentrations of DHM did not affect the mean light intensity in the limnocorrals. The extinction coefficients (k, Table 1) in the moss–peat limnocorrals were similar to the controls. After 1 yr in the limnocorral (OM82-8) the moss–peat material no longer released humic material, P, or N in quantities sufficient to raise concentrations above the controls (Table 4).

Immediately after the moss–peat addition, chlorophyll and suspended C values in the low-moss–peat limnocorrals (LM81-2 and LM82-3) were similar to or greater than those in the controls (Fig. 3). However, within 3–4 wk of the addition, biomass was less in the low-moss–peat limnocorrals than in the controls. Mean chlorophyll and suspended C concentrations in the low-moss–peat limnocorrals were 30% less than those of the controls each year (Table 4). The seasonal pattern of integral productivity was similar to that of biomass (Fig. 4). Mean integral productivity in the low-moss–peat limnocorrals (LM81-2, LM82-3) was 20–30% less than in the newly established controls (Table 4).

In the 1-yr-old moss–peat limnocorral (OM82-8), biomass was similar to both the new and old controls (C82-6, OC82-5, Table 4). Mean integral primary productivity in this limnocorral (OM82-8) was similar to the 1982 control (C82-6) and 30% greater than integral productivity in the 1-yr-old control (OC82-5, Table 4).

In the high-moss–peat limnocorral (HM82-4), mean chlorophyll and suspended C concentrations were 1.5 and 2 times the control values, respectively (Table 4). By the summer's end, biomass in the high-moss–peat limnocorral appeared to be declining (Fig. 3). Primary productivity was not measured routinely in this limnocorral; however, on the two occasions it was measured, integral productivity was about double that in the low-moss–peat limnocorral (LM82-3) (Table 4; Fig. 4).

Moss–peat material reduced the occurrence and severity of P deficiency in the limnocorrals. According to indicators of P deficiency (Table 4), both the low-moss–peat limnocorrals (LM81-2, LM82-3) and the high-moss–peat limnocorral (HM82-4) were much less P deficient than the controls. In the 1-yr-old moss–peat limnocorral (OM82-8), the degree of P deficiency was similar to the control. There were no signs of N deficiency in any of the moss–peat or control limnocorrals (Guildford 1985).

The elevated concentrations of TDP and TDN in the lim-

TABLE 4. Seasonal means of moss–peat limnocorral data. A_{455} = absorbance at 455 nm; TDN = total disolved nitrogen; TDP = total dissolved phosphorus; Pase = alkaline phosphatase activity (μmol *o*-methylfluoroscein phosphate hydrolyzed·h^{-1}·μg ATP^{-1}); Pdebt = estimated μmol phosphate removed in 24 h of darkness·μg ATP^{-1}). See Table 3 for interpretation of other nutrient deficiency indicators and units for other headings. ND = not done.

Limnocorral	Treatment	A_{455} (nm)	TDN (μmol·L^{-1})	TDP (μmol·L^{-1})	I.P. (mg C·m^{-2}·d^{-1})	I_{24} (E·m^{-2}·d^{-1})	I_k (E·m^{-2}·d^{-1})	Chl*a* (μg·L^{-1})	Susp. C (μg·L^{-1})	Pase[a]	Pdebt[b]	P/C (μg·mg^{-1})	N/P (μg·μg^{-1})
1981													
C81-1	Control	0.045	22.6	0.23	500	21.1	17.1	3.9	719	1.226	2.635	14	16
LM81-2	Moss–peat (63 kg)	0.070	26.0	0.42	399	16.2	20.2	2.7	496	0.061	0.120	27	7
1982													
C82-6	Control	0.062	22.6	0.32	290	18.7	19.3	2.2	909	0.079	ND	11	20
LM82-3	Moss–peat (52 kg)	0.079	24.2	0.52	212	15.1	23.3	1.6	645	0.007	ND	15	15
HM82-4	Moss–peat (104 kg)	0.100	27.5	0.55	436[c]	16.5	19.0	4.4	1442	0.004	ND	17	13
OC82-5	Old control (1 yr old)	0.053	21.8	0.29	217	19.4	18.9	2.1	885	0.047	ND	12	17
OM82-8	Old moss–peat (63 kg)	0.055	23.7	0.36	296	19.8	20.4	2.5	913	0.083	ND	12	17

[a] 0.02–0.10 = moderate, >0.10 = severe P deficiency.
[b] >0.500 = P deficiency.
[c] Based on two values.

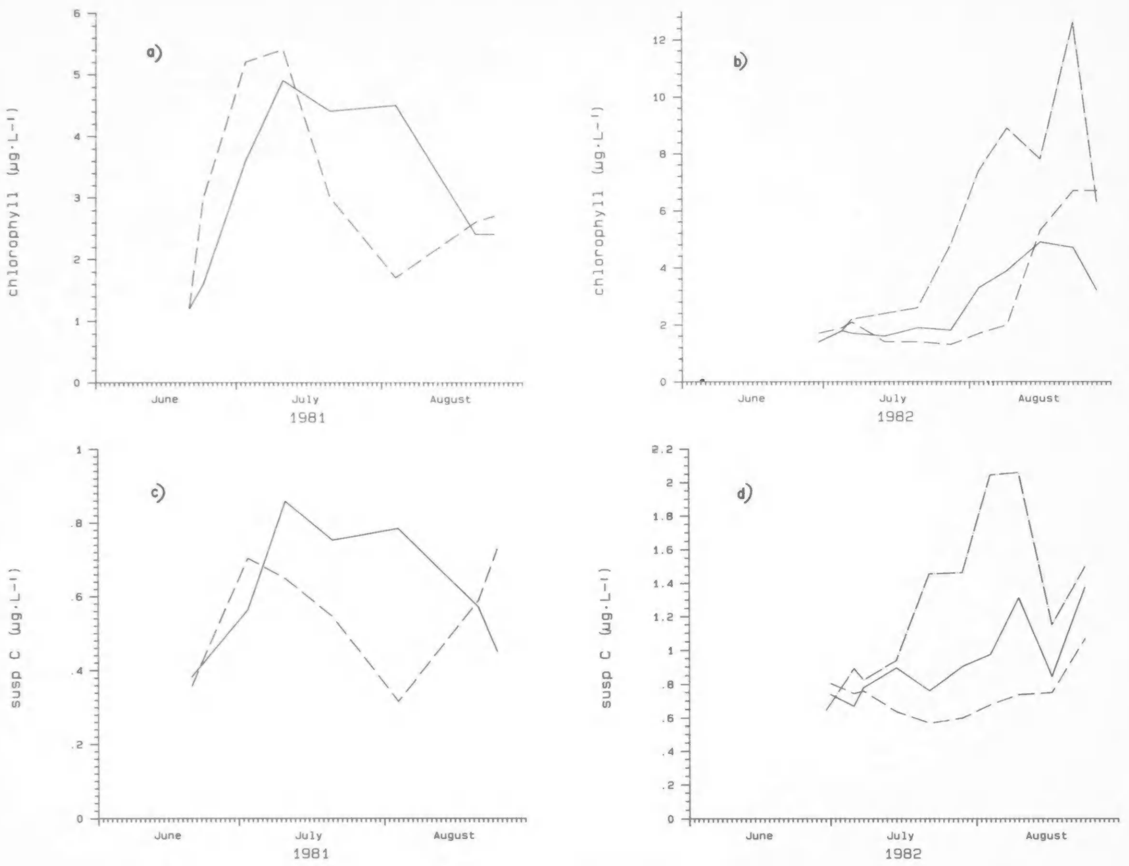

FIG. 3. Seasonal pattern of chlorophyll *a* and suspended carbon in the 1981 and 1982 control (—), low-moss–peat (- - -), and high-moss–peat (- ·-) limnocorrals.

nocorrals receiving moss–peat (Table 4) showed that this material was a source of dissolved P and N. Initially in the low-addition limnocorrals (LM81-2, LM82-3), biomass was higher as a result of increased nutrients (Fig. 3). After 2–3 wk, biomass and integral primary productivity in the low-moss–peat limnocorrals were significantly less than in the controls, but nutrient status indicators showed that P was not limiting the algae. However, in the high-moss–peat limnocorral (HM82-4), the increased nutrients resulted in higher biomass for most of the summer. The increased loading of nutrients in the high-moss limnocorral (HM82-4) may have prolonged the period of enhanced productivity and biomass beyond that seen in the low-moss limnocorrals.

Some factor other than P or N was limiting algal productivity and biomass in the moss–peat limnocorrals. DHM absorbs light selectively at 350–500 nm (Wetzel 1975), which is the range of the major peak in absorption spectra of both chlorophyll and carotenoids. Arvola (1984) showed that, in a lake containing high concentrations of DHM, primary productivity was negatively affected because of reduced light penetration. Although I_{24} in the moss-peat limnocorrals (LM81-2, LM82-3) was slightly less than in the controls, it is unlikely that decreased light intensity or changed light quality was the limiting factor because I_{24} was low all summer, while biomass and integral productivity were low only in the last half of the summer. Koenings and Hooper (1976) and Francko and Heath (1983) have demonstrated that DHM binds PO_4 via $Fe(OH)_3$ and suggested that high concentrations of DHM may cause P limitation. In the moss–peat limnocorrals, there was clearly a correlation between DHM and TDP (Fig. 5), but the nutrient status measurements show that P was not limiting. In this situation, Fe itself or some other metal bound by DHM may have limited phytoplankton.

Jackson and Hecky (1980), studying lakes and reservoirs in the boreal forest of northern Manitoba, including SIL, found an inverse correlation between primary productivity and humic, colloidal, hydrated-ferric-oxide complexes. They hypothesized that humic matter binds Fe, making it unavailable to algae. Sakamoto (1971) also found evidence for Fe limitation in the presence of humic matter. He found that the addition of humic matter alone to lake water reduced primary production but the addition of humic matter plus Fe and trace metals stimulated primary production.

In the moss–peat limnocorral studied for 2 yr (OM82-8), the inhibitory effect of the moss–peat material disappeared after 1 yr. Limnocorral water was allowed to circulate with outside

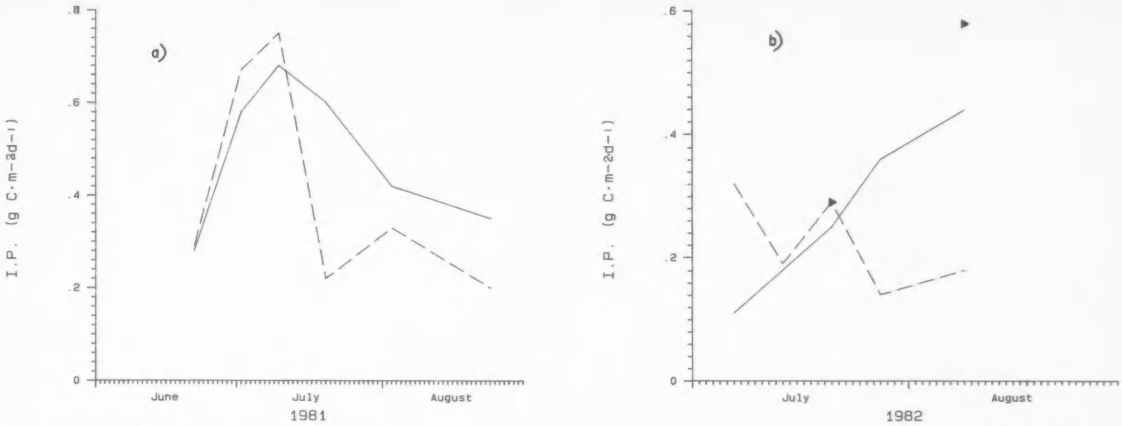

FIG. 4. Seasonal pattern of integral primary productivity in the control (—) and low-moss–peat (- - -) limnocorrals. The two values for the high-moss–peat limnocorral are denoted by ▶.

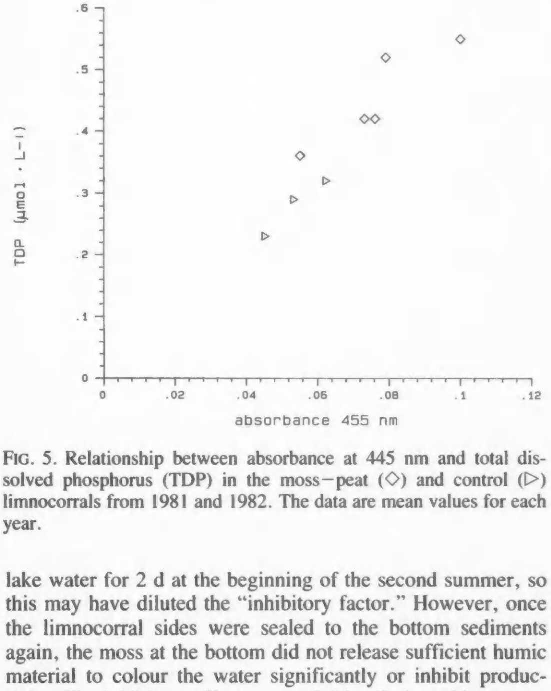

FIG. 5. Relationship between absorbance at 445 nm and total dissolved phosphorus (TDP) in the moss–peat (◇) and control (▷) limnocorrals from 1981 and 1982. The data are mean values for each year.

lake water for 2 d at the beginning of the second summer, so this may have diluted the "inhibitory factor." However, once the limnocorral sides were sealed to the bottom sediments again, the moss at the bottom did not release sufficient humic material to colour the water significantly or inhibit productivity. The inhibitory effect was probably eliminated by a combination of photodegradation of DHM (Stewart and Wetzel 1981; Francko and Heath 1979), by bacterial mineralization of the moss–peat material, and by flushing.

Enrichment Experiments

The enrichment experiments were designed to test three hypotheses regarding the moss–peat limnocorrals: (1) Fe or some other metal bound by the DHM in the moss–peat limnocorrals was limiting algal productivity and biomass; (2) the reduction in light intensity and/or change in light quality due to selective adsorption by DHM was the limiting factor in the moss–peat limnocorrals; (3) in order for the inhibitory affect of the moss–peat material to occur, the moss–peat material must be in physical contact with the water.

The initial and final values for chlorophyll *a*, C uptake, and alkaline phosphatase activity for the three enrichment experiments are given in Table 5. Data for the intervening days of the experiments fell along the trend between initial and final values. In each experiment the largest response occurred when water from the moss–peat limnocorral (LM81-2 in 1981, LM82-3 in 1982) was enriched with Fe and EDTA. There was a smaller increase in chlorophyll and C uptake in the 1981 control limnocorral water (C81-1) in response to Fe and EDTA and to EDTA alone and none in C82-6 in 1982.

The inclusion of moss–peat material dredged from the bottom of the moss–peat limnocorral (LM82-3) also increased chlorophyll concentration, C uptake, and alkaline phosphatase activity in water from the moss–peat limnocorral above the levels with no enrichment, but not to the degree of Fe and EDTA.

The pattern was similar in the in situ incubation (Table 5); the largest response was to Fe and EDTA in the container suspended at the surface. At 0.8 m, only the container enriched with Fe and EDTA responded. There was little difference between the unenriched container suspended at 0.8 m and the screen-covered unenriched container suspended at the surface.

The results support, but do not prove, the hypothesis that the moss–peat limnocorrals were limited by Fe or some other metal bound by humic matter. Either the Fe itself added to the water or some reaction with the EDTA increased biomass and productivity in the water from the moss–peat limnocorrals.

Anderson and Morel (1982), using labelled ^{59}Fe showed, that EDTA chelated Fe so tightly that it was unavailable to Fe-limited algae. However, in the light, EDTA broke down rapidly, ^{59}Fe became available, and the Fe-limited algae were stimulated. This may have been the mechanism by which EDTA stimulated the algae in the enrichment experiments.

It was expected that if DHM significantly reduced light penetration in the moss–peat limnocorrals, an increase in chlorophyll concentration and productivity would be observed in the untreated carboy suspended at the surface of the moss–peat limnocorral. At the end of the 9-d in situ experiment, there was

TABLE 5. Initial and final values for chlorophyll (μg·L^{-1}), photosynthesis (mg C·m^{-3}·h^{-1}), and alkaline phosphatase activity (μmol P·h^{-1}·μg ATP^{-1}) in three enrichment experiments. See Table 2 for enrichment concentrations.

		Control limnocorral (C81-1)				Moss–peat limnocorral (LM81-2)			
Experiment I (1981)	Day	None	Trace metals	FeEDTA	EDTA	None	Trace metals	FeEDTA	EDTA
Chlorophyll	4	1.4	1.1	1.0	1.3	2.0	1.3	2.6	1.9
	7	1.4	1.1	1.7	1.5	3.2	2.7	5.1	4.1
Photosynthesis	4	3.8	3.3	3.8	3.2	7.4	6.0	5.7	5.3
	7	3.4	3.6	5.2	4.2	9.0	9.3	11.1	10.6
Alkaline phosphatase	4	0.107	0.127	0.310	0.162	0	0.023	0.015	0
	7	0.214	0.227	0.165	0.240	0.153	0.144	0.110	0.115

		Control limnocorral (C82-6)			Moss–peat limnocorral (LM82-3)		
Experiment II (1982)	Day	None	FeEDTA	Moss	None	FeEDTA	Moss
Chlorophyll	1	2.6	2.7	2.9	1.4	1.6	2.5
	6	1.1	1.3	4.2	3.0	11.4	10.8
Photosynthesis	1	5.1	8.7	7.5	3.7	3.6	5.2
	6	1.1	1.9	3.2	4.0	31.8	18.7
Alkaline phosphatase	1	0.125	0.195	0.119	0.002	0.005	0.001
	6	0.086	0.134	0.077	0	1.714	0.523

		In situ moss–peat limnocorral (LM82-3)						
		Surface				0.8 m		
Experiment III (1982)	Day	None	FeEDTA	Moss	Screen	None	FeEDTA	Moss
Chlorophyll	0	1.5	1.6	2.0	1.7	1.6	1.9	1.6
	9	2.5	7.3	3.4	2.2	2.2	4.7	2.6
Photosynthesis	0	7.0	7.4	8.1	6.7	6.7	9.3	6.9
	9	7.3	29.7	9.7	5.3	4.6	13.9	5.3
Alkaline phosphatase	0	0.026	0.019	0.011	0.040	0.015	0.026	0.029
	9	0.009	0.280	0.015	0.009	0.004	0.041	0.009

a slightly higher chlorophyll concentration and higher rates of primary productivity and alkaline phosphatase activity in the untreated carboy at the surface compared with the carboy suspended at 0.8 m. There was a much greater increase in the parameters measured in the two carboys enriched with Fe and EDTA suspended at both depths. This suggests that light intensity was not a limiting factor. It was only when the limiting factor was relieved by Fe and EDTA that a significant response to light was observed. Similarly, if light quality due to selective absorption by DHM was a limiting factor in the moss–peat limnocorrals, it was expected that the chlorophyll concentration and primary productivity would be greater in the screen-covered container suspended at the surface than in the carboy suspended at 0.8 m. There was no difference in chlorophyll *a* concentration but primary productivity was slightly higher at the surface.

When moss–peat material dredged from the moss–peat limnocorral was incubated with the water in flasks in the laboratory and in large containers at the surface of the limnocorrals, chlorophyll concentration and primary productivity increased. This effect is opposite to that expected because the moss–peat material caused inhibition in the limnocorral. The fact that little stimulation occurred in the carboy suspended at 0.8 m and that the response in the flask incubated in the laboratory was much greater than the response in situ at the surface suggested that the reason for the stimulation by the moss–peat material might be related to light. Humic matter is broken down by sunlight (Stewart and Wetzel 1981; Francko and Heath 1983). Therefore, Fe may have been released from humic matter in a manner similar to that described for EDTA and Fe by Anderson and Morel (1982). The fact that moss–peat material stimulated rather than inhibited growth in these enrichment experiments indicates that moss–peat material did not release toxic substances (e.g. metals or phenolic compounds) to cause the observed inhibition.

Conclusion

The responses, observed in the limnocorral experiments, to the two main types of flooded shoreline material from SIL were similar to the actual responses observed in SIL after flooding, where P deficiency was relieved in all parts of lake but primary productivity did not generally increase (Hecky and Guildford 1984). The limnocorral experiments, if done before impoundment, would have made valid predictions about the effects on water quality and primary productivity whereas the simple application of P loading models, which assume P limitation of algae, would have predicted increased algal productivity.

Limnocorral experimentation should be considered an effective approach to predicting the effect of different flooded materials on algal productivity and nutrient status. Such experiments, using realistic loading rates, should be done for new reservoirs to determine if P loading models are appropriate for predicting primary productivity in the non-steady-state conditions immediately subsequent to impoundment. In northern boreal reservoirs such as SIL, where a substantial amount of clay is brought into suspension by shoreline erosion or when moss−peat is flooded, P will not limit algal populations after impoundment.

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Latent Effects of Pulse Exposure to Aluminum and Low pH on Size, Ionic Composition, and Feeding Efficiency of Lake Trout (*Salvelinus namaycush*) Alevins

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Gunn, J. M., and D. L. G. Noakes. 1987. Latent effects of pulse exposure to aluminum and low pH on size, ionic composition, and feeding efficiency of lake trout (*Salvelinus namaycush*) alevins. Can. J. Fish. Aquat. Sci. 44: **1418–1424.**

No mortality of lake trout (*Salvelinus namaycush*) embryos occurred during 5 d of exposure to aluminum concentrations of 0, 100, and 200 $\mu g \cdot L^{-1}$ in low-pH (pH 5.0) water or during 21- and 32-d recovery periods, but alevins from the high-Al exposure were smaller in length, had less calcified skeletons, had reduced (18−22%) whole-body concentrations of Ca^{2+} and K^+, and were less effective predators on *Daphnia magna*. These latent effects of pulse exposure to acidic Al-rich water may help explain the disappearance of lake trout populations from lakes with relatively high ambient pH.

Après 5 j d'exposition à des concentrations de 0, 100 et 200 $\mu g \cdot L^{-1}$ d'aluminium en milieu à faible pH (pH 5,0) ou pendant les périodes de récupération de 21 et 32 j, les auteurs ont observé l'absence de mortalité chez les embryons de touladi (*Salvelinus namaycush*). Toutefois, les alevins exposés à une concentration élevée de Al étaient moins longs, leur squelette était moins calcifié et les concentrations corporelles totales de Ca^{+2} et K^+ étaient réduites (18−22 %); de plus, ils sont devenus moins efficaces à la capture de *Daphnia magna*. Ces incidences latentes d'une exposition pulsée à de l'eau acide riche en Al peut expliquer la disparition des populations de touladi dans les lacs où le pH ambiant est relativement élevé.

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Episodic acidification of surface water is a widespread phenomenon in areas receiving high deposition of acidic precipitation (Jeffries et al. 1979; Henriksen et al. 1984; Kelso et al. 1986). These short-term chemical changes are usually associated with snowmelt or periods of heavy rain. In addition to acid inputs, large quantities of aluminum are leached from watershed soils and carried in solution to receiving lakes and streams (Cronan and Schofield 1979). The combination of high levels of H^+ and inorganic Al in dilute runoff water can have severe effects on fish and other aquatic biota (Leivestad and Muniz 1976; Driscoll et al. 1980; Henriksen et al. 1984).

Knowledge of the immediate and longer term effects of episodic acidification on fish survival may help explain the present discrepancies between laboratory and in situ findings. For example, natural lake trout (*Salvelinus namaycush*) populations decline when whole-lake average pH drops below 5.5−5.6 (Schindler et al. 1985; Beggs and Gunn 1986). In contrast, laboratory tests show little effect on the short-term survival of sensitive early life stages of lake trout until pH drops below 4.8 (Hutchinson et al. 1987). The laboratory-derived pH threshold does not substantially increase when associated chemical parameters such as Ca and Al are manipulated to better match field conditions (Hutchinson et al. 1987). However, lakes with average pH < 6.0 are known to experience severe depressions in pH and elevated levels of Al (LaZerte 1984; Gunn and Keller 1984; Kelso et al. 1986), and these short-term chemical changes may be responsible for increased mortality of fish in such lakes (Siddens et al. 1986).

Lake trout embryos within the rubble of shallow nearshore spawning sites are highly vulnerable to acidic runoff water (Gunn and Keller 1984). Embryos are relatively immobile, and acute mortality may occur as acidic runoff water inundates the spawning substrate. In toxicity tests conducted at a lake trout spawning site in Whitepine Lake, Ontario, Gunn and Keller (1984) found that mortality of lake trout embryos began during periods of runoff, but ceased after ambient chemical conditions returned. Overall mortality was low (18%) and surviving alevins appeared healthy, but no studies of possible latent effects were carried out. While a great deal of information is now available on acute effects of low pH and Al exposure on several species of fish (Fromm 1980; Wood and McDonald 1982, 1987), latent effects from pulse exposure have received little attention in either laboratory or field studies.

In this study, we conducted a laboratory simulation of chemical conditions typical of a Precambrian Shield lake during snowmelt (Gunn and Keller 1984) to examine latent effects on early life stages of lake trout. Embryos (fish dependent only on yolk material for nutrition) were exposed to sublethal levels of H^+ and inorganic Al for 5 d and then transferred to control water until they became free-swimming alevins (transition stage between endogenous and exogenous feeding). Latent ef-

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TABLE 1. Measured physical and chemical conditions during 5 d of exposure of lake trout embryos to low pH (5.0) and a range of Al concentrations. Means and 95% confidence intervals (in parentheses) are given (means and ranges given for pH).

			Nominal Al concentrations at pH 5.0		
	N	Control	0 $\mu g \cdot L^{-1}$	100 $\mu g \cdot L^{-1}$	200 $\mu g \cdot L^{-1}$
pH	15	6.9 (6.8–7.0)	5.1 (5.0–5.2)	5.0 (5.0–5.1)	5.1 (5.0–5.2)
Total Al ($\mu g \cdot L^{-1}$)	15	4 (1)	5 (1)	101 (4)	199 (9)
Inorg. Al ($\mu g \cdot L^{-1}$)	5–14	na	6 (4)	107 (6)	194 (9)
Ca^{2+} ($mg \cdot L^{-1}$)	15	1.54 (0.07)	1.58 (0.03)	1.53 (0.02)	1.63 (0.02)
Na^{+} ($mg \cdot L^{-1}$)	15	1.50 (0.03)	1.38 (0.03)	1.39 (0.04)	1.42 (0.03)
Cond. ($\mu S \cdot cm^{-1}$ at 25°C)	15	19.3 (0.1)	24.1 (1.0)	26.3 (0.6)	28.0 (0.1)
Hardness ($mg \cdot L^{-1}$ as $CaCO_3$)	15	5.3 (0.2)	5.1 (0.2)	5.1 (0.1)	5.6 (0.2)
Temp (°C)	15	4.5 (0.1)	4.7 (0.1)	4.6 (0.1)	4.4 (0.1)

fects examined included changes in final size, degree of calcification of the alevin skeleton, and feeding efficiency. We also measured electrolyte concentrations in the alevins to determine if pulse exposure to sublethal levels of H^+ and Al would cause physiological effects that persisted throughout the recovery period. Whole-body electrolytes had previously been shown to be sensitive indicators (Wood and McDonald 1982; Lacroix et al. 1985) of ionoregulatory failure, the principal physiological response of fish to acid (McDonald 1983) or acid plus Al (Neville 1985; Wood and McDonald 1987) in low-Ca water.

Materials and Methods

Gametes from several adult lake trout from Lake Manitou (45°60′N, 82°00′W) were pooled to provide fertilized eggs for this study. The eggs were kept at a hardwater (266 $mg \cdot L^{-1}$ as $CaCO_3$) hatchery for 52 d at 7.5°C before transfer to the soft water (5 $mg \cdot L^{-1}$ as $CaCO_3$) of the laboratory. Separate lots of eggs were reared at different temperatures (0.5–6°C) to alter developmental rate of embryos. Two groups of embryos that differed widely in accumulated thermal units (599; 712°C degree-days) were used to test the effect of H^+ and Al. The experiment began after embryos had been held for 75 d in soft water. Both groups of embryos were acclimated ($1° \cdot d^{-1}$) to a common test temperature (5°C) and maintained at this temperature for 10 d prior to the test. Hatching occurred several weeks before the experiments (at ~450–500 degree-days), but for convenience the groups are hereafter referred to as "early" and "late" embryos. Initial mean total lengths of embryos and dry weights of yolk reserves were as follows: early embryos — 23.4 ± 0.5 (95% CI) mm, 13.9 ± 2.6 mg; late embryos — 25.2 ± 0.9 mm, 10.5 ± 2.3 mg.

The soft water used for holding embryos, and as control water during H^+ and Al tests, consisted of an ultraviolet-sterilized mixture of deionized and dechlorinated Toronto tap water (98/2 mixture). The concentrations of major ions in the softwater were as follows: Ca^{2+}, 1.80 $mg \cdot L^{-1}$; Na^+, 1.38 $mg \cdot L^{-1}$; K^+, 0.12 $mg \cdot L^{-1}$; Mg^{2+}, 0.39 $mg \cdot L^{-1}$; Cl^-, 2.07 $mg \cdot L^{-1}$. The following metals were at <5 $\mu g \cdot L^{-1}$: Mn, Cu, Ni, Pb, Cd, Zn, and Al. Test solutions were prepared by adding $Al_2(SO_4)$ and H_2SO_4 to the soft water. Vigorous aeration to drive off free CO_2 and repeated titrations with 0.02 *N* H_2SO_4 were used to produce stable acidic pH conditions (pH 5.0) for each of the three Al concentrations (0, 100, and 200 $\mu g \cdot L^{-1}$). Test solutions were kept at 5°C and aerated continuously for 10–24 h prior to use.

Exposure of embryos to H^+ and Al occurred in 40-L static tanks. There were three replicate tanks per treatment including controls. Each tank held 20 randomly selected early and late embryos in two separate 9.5-cm-diameter floating pens. Tanks were continuously aerated and were kept in the dark except during water sampling, water changes, and mortality checks. All solutions were changed (100%) daily. Floating pens were moved carefully and quickly between replicates during water changes. Samples were collected immediately before and after the daily water changes and showed <5% loss of aqueous inorganic Al through precipitation or adherence to surfaces. The low loading rate (~0.01 g dry $wt \cdot L^{-1}$) of nonfeeding fish and the low levels of dissolved organic matter (0.2 $mg \cdot L^{-1}$) in the test water were presumably responsible for the good agreement between nominal and measured inorganic Al concentrations in the static tanks (Table 1).

Temperature (±0.05°C) was measured daily in each tank using a NBS standard Hg thermometer. Daily water samples were collected in polystyrene bottles from each tank for analysis of pH (±0.01), specific conductance at 25°C (±0.3 $\mu S \cdot cm^{-1}$), and total hardness (±0.3 $mg \cdot L^{-1}$ as $CaCO_3$). All pH readings were made within 12 h of sample collection using Radiometer PM82 meter equipped with a low ionic strength electrode (Radiometer GK2401C). Water samples were submitted to Ontario Ministry of the Environment (OMOE) laboratories for analysis of Na^+, Ca^{2+}, and total Al by atomic absorption spectroscopy (OMOE 1981) and analysis of inorganic Al by the pyrocatechol violet colorimetric method (Rogeberg and Henriksen 1985). A summary of the measured physical and chemical characteristics of the control and treatment water used during the exposure period is presented in Table 1.

At the end of the 5-d exposure period, all fish were transferred to control water at 4.9 ± 0.1°C until embryos became free-swimming alevins. In early embryos the recovery period lasted 32 d. In the more advanced late embryos the recovery period was 21 d. No fish were fed during the recovery period. At the end of the recovery period, replicates were pooled and a random sample of 30 early and 20 late embryos drawn for length and weight measurements. Total length of fresh specimens was measured with calipers. Dry weights were determined after oven drying at 65°C for 24 h.

Alevins were analyzed for Na^+, Cl^-, K^+, Mg^{2+}, Ca^{2+}, and Al by neutron activation. Samples were irradiated in a neutron flux of $\sim 5 \times 10^{12}$ $n \cdot cm^{-2} \cdot s^{-1}$ for 200 s. They were immediately transferred to preweighed nonirradiated vials, and after a 240-s delay were counted for 500 s with a gamma ray spec-

TABLE 2. Effects of 5 d of exposure of lake trout embryos to pH 5.0 plus 0, 100, an
embryos) and 32 d (early embryos) of recovery in control water (pH 7.0, no Al). Mea
same letter(s) indicate that group means are not significantly different (Duncan's mu

	Group	N[a]	Initial[b]	Control (pH 7.0, no Al
Total length (mm)	Early	30	na	27.1 (0.4) a
	Late	30	25.8 (0.4) a	27.2 (0.6) b
Dry weight (mg)	Early	30	na	20.2 (1.2) a
	Late	30	22.6 (1.4) a	16.7 (1.5) b
Ca^{2+} (meq · 100 g^{-1})	Early	20	na	27.64 (1.71) a
	Late	20	13.70 (0.65) a	29.52 (1.30) b
K^{+} (meq · 100 g^{-1})	Early	20	na	40.93 (3.55) a
	Late	20	23.91 (1.25) a	40.80 (1.88) b
Na^{+} (meq · 100 g^{-1})	Early	20	na	31.80 (1.99) ab
	Late	20	23.33 (1.45) a	30.62 (1.61) b
Mg^{2+} (meq · 100 g^{-1})	Early	20	na	10.20 (0.69) ab
	Late	20	10.45 (0.63) ac	11.07 (0.62) ab
Cl^{-} (meq · 100 g^{-1})	Early	20	na	25.30 (1.93) a
	Late	20	18.42 (0.76) a	25.35 (1.40) b

[a] N = 10 for 200 μg Al · L^{-1} and control groups for ion data of early embryos; N
[b] Embryos collected from control water on day 4 of 5-d exposure. No initial samp
[c] Difference (%) = (control − 200 μg Al · L^{-1} group)/(control) × 100.

100, and 200 μg Al·L^{-1} on size and whole-body ionic composition following 21 d (late
Al). Means and 95% confidence intervals (in parentheses) are given. Data followed by the
can's multiple range test, $p \leq 0.05$).

ntrol 0, no Al)	Al (μg·L^{-1}) 0	100	200	Difference[c] (%)
.4) a	27.0 (0.4) a	26.8 (0.4) a	26.0 (0.3) b	4.0
.6) b	27.2 (0.5) b	26.3 (0.6) c	26.2 (0.5) c	3.7
.2) a	20.5 (1.0) a	21.3 (1.0) a	20.2 (0.9) a	0
.5) b	17.4 (1.3) b	16.2 (1.6) b	17.5 (1.5) b	0
(1.71) a	26.95 (1.63) a	22.48 (1.36) b	21.41 (2.34) b	22.5
(1.30) b	28.64 (1.32) bc	27.07 (1.82) c	23.38 (1.22) d	20.8
(3.55) a	40.03 (2.20) a	32.49 (1.82) b	33.51 (4.08) b	18.1
(1.88) b	38.88 (2.08) bc	36.71 (3.39) c	33.13 (2.38) d	18.8
(1.99) ab	32.49 (1.49) a	28.99 (1.58) c	29.45 (2.91) bc	7.4
(1.61) b	31.82 (1.37) b	31.65 (1.63) b	28.47 (1.57) c	7.0
(0.69) ab	11.03 (0.63) a	9.22 (0.74) b	9.71 (0.87) b	4.8
(0.62) ab	11.31 (0.60) b	11.40 (0.73) b	10.04 (0.39) c	9.3
(1.93) a	25.43 (1.21) a	22.36 (1.02) b	22.37 (1.82) b	11.6
(1.40) b	25.04 (1.26) b	25.67 (1.94) b	22.55 (1.33) c	11.0

ryos; $N = 20$ for the other groups.
tial samples are available for the early embryos.

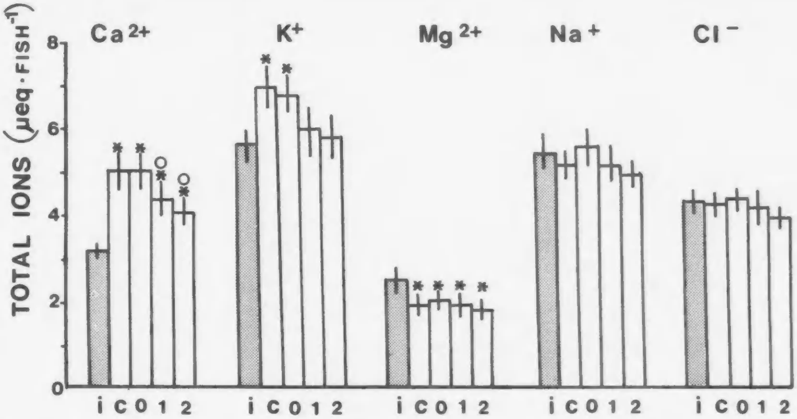

FIG. 1. Changes in the electrolyte content of late embryos during the recovery period. Initial embryos (i, shaded bars) were collected from the control water on day 136 (day 4 of 5-d exposure). Exposures: c = control, pH 7.0; 0 = pH 5.0, no Al; 1 = pH 5.0, 100 μg Al·L^{-1}; 2 = pH 5.0, 200 μg Al·L^{-1}. Alevins were collected on day 158, 21 d after return to control water from exposures. Means and 95% confidence intervals (vertical bars) indicated. *Significantly ($p < 0.05$) different from initial; °significantly different from both the initial and control.

trophotometer. Twenty dried (65°C, 24 h) alevins from each treatment were analyzed individually. Because of interference by ^{31}P within the spectral peak of ^{28}Al, samples were digested with acid and analyzed for total P by inductive coupled plasma emission spectroscopy (ICP). Initial Al values were then corrected according to measured levels of P. Accuracy of analytical procedures was tested using NBS standard material (oyster tissue, No. 1566). Observed values for Ca^{2+}, K^{+}, and Cl^{-} varied within 5% of certified values. Mg^{2+} and Na^{+} varied by 9.5 and 6.1%, respectively.

Ten specimens from each of the treatments were cleared with trypsin and the skeletons stained (alizarin red) for calcium phosphate in calcified bone using the methods described in Potthoff (1984). The degree of calcification was scored (0−4) according to the amount of red stain present on each of the five rayed fins. The sum of the scores (maximum 20) was used as a measure of the degree of skeletal calcification for each fish. All fish were viewed on the right side through a stereomicroscope. The presence of ossified teeth was also noted. Only specimens from the late embryo group could be used for assessment of skeletal Ca. The early embryo group became overstained, a frequent problem with this procedure (Potthoff 1984).

Feeding trials were performed with emergent alevins from the early embryo group on days 31 and 32 postexposure. Laboratory cultured *Daphnia magna*, of a size (length 1.28 ± 0.04 mm, dry wt 11.3 ± 1.5 μg) edible by all treatment groups, were used as prey. Twenty live daphnia were placed in glass jars containing 3.5 L of control water. Each day, 40 jars (10 replicates per treatment) were arranged in a stratified random pattern within a large (70 × 170 × 20 cm) open 5°C water bath. Light from overhead fluorescent bulbs provided illumination at the water surface in the open jars of about 10 mE·m^{-2}·s^{-1}. Some warming of the water in the jars occurred during the feeding trials (final temperature 6.2 ± 0.1°C); however, it was assumed that any temperature effects would apply equally to all treatments. The sides of the jars were covered with translucent paper to prevent alevins from seeing each other. A trial consisted of introducing a single randomly selected alevin into each jar. After 5 h, alevins were removed and daphnia remaining in the bottles were counted. Each alevin was used only once in the feeding trials.

Blind procedures were used throughout to eliminate biases, particularly for assigning scores to stained specimens and measuring lengths. Lengths, weights, and ion concentrations were compared by ANOVA ($p < 0.05$) followed by Duncan's multiple range test. No transformations were required. Feeding rates and scores assigned to stained specimens were compared using nonparametric procedures (Kruskal−Wallis).

Results

No mortality occurred during the 5-d exposure period or during the 21- and 32-d recovery periods in any of the treatment groups. None of the embryos or later alevins displayed erratic swimming behaviour or mucus accumulation around the mouth and/or gills.

Exposure to Al in acidic water (pH 5.0) reduced the growth in body length of embryos during the recovery period (Table 2). Postexposure growth rates of embryos from the 100 μg Al·L^{-1} (0.023 mm·d^{-1}) and 200 μg Al·L^{-1} (0.018 mm·d^{-1}) treatments were reduced by 63 and 71%, respectively, as compared with control embryos (0.063 mm·d^{-1}). Both early and late embryos were similarly affected by Al. The final size of alevins from the 200 μg Al·L^{-1} treatment groups was reduced, relative to control fish, by 4.0% in early embryos and 3.7% in late embryos (Table 2). Exposure to pH 5.0, without Al, produced no significant effects on growth. Alevins from the 100 and 200 μg Al·L^{-1} treatment groups had visibly greater amounts of remaining yolk material and did not differ in whole-body (includes yolk) dry weight from control fish (Table 2), indicating that exposure to Al produced the length reductions through a delay in the use of yolk material for

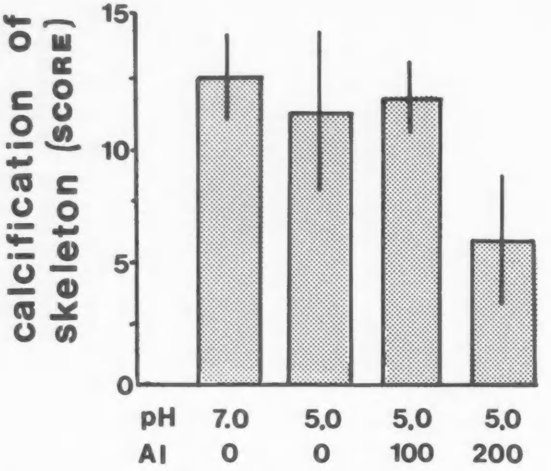

FIG. 2. Skeletal Ca in lake trout alevins, pulse exposed as embryos to the four indicated treatment conditions. Scores were assigned according to the amount of alizarin red stain present on the fins (see text for details). Means and 95% confidence intervals (vertical bars) indicated.

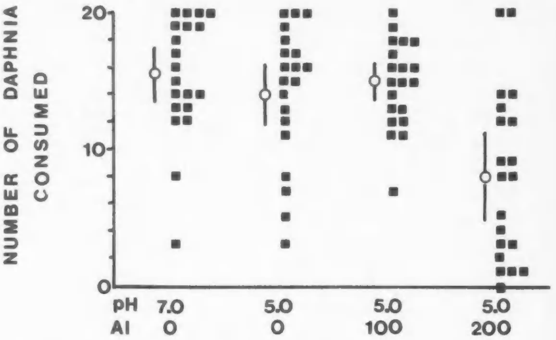

FIG. 3. Effects of prior exposure to pH 5.0 plus 0, 100, and 200 μg $Al \cdot L^{-1}$ on feeding rate (number of daphnia eaten in 5 h) of lake trout alevins. ■, individual fish; ○, group means; vertical bars, 95% confidence intervals.

growth of somatic tissues, rather than as a result of an increase in total energy expenses.

Exposure of embryos to Al during the simulated runoff event produced significant reductions in the whole-body concentrations of major ions in the resulting alevins (Table 2). Exposure to pH 5.0 alone produced no significant effects on ion content. Relative to control fish, exposure to Al produced greatest reductions in Ca^{2+} (20–22%) and K^+ (18–19%), followed by Cl^- (11%), Na^+ (7%), and Mg^{2+} (5–10%). Early embryos appeared to be more sensitive to Al, with both the 100 and 200 $\mu g \cdot L^{-1}$ treatments producing significant differences in ion concentrations. In the alevins exposed as late embryos, only the 200 μg $Al \cdot L^{-1}$ treatment group differed from the control.

During the yolk absorption period, embryos were undergoing whole-body compositional changes through an active uptake of some ions from the water as well as a net loss of others (Fig. 1). The total content of Ca^{2+} and K^+ in control embryos increased by 56.9 and 24.0%, respectively, during the 22-d holding period. Mg^{2+}, an element in excess supply within the yolk (Peterson and Martin-Robichaud 1986), showed a 23.4% net loss over the same period. No significant changes in the total content of Na^+ and Cl^- occurred. Embryos exposed to Al displayed significant changes in these typical ion flux patterns. Net accumulation of K^+ was eliminated, while Ca^{2+} accumulation was reduced to 37.2 and 27.8%, respectively, in the 100 and 200 μg $Al \cdot L^{-1}$ treatment groups. Mean values of total Na^+ and Cl^- were also lowest in the high-Al treatment groups, but the differences relative to the initial sample, or the control fish, were not significant (Fig. 1).

The clearing and staining procedures demonstrated that delayed calcification of the alevin skeleton occurred in Al-stressed fish (Fig. 2). The 200 μg $Al \cdot L^{-1}$ treatment group was readily distinguishable from other treatment groups by the almost complete lack of stain on dorsal and pelvic fins and the low number of completely stained neural and hemal arches. Jaws and teeth were generally well stained with alizarin red in all specimens.

No long-term accumulation of Al was detected in the specimens analyzed by neutron activation. All alevins had Al concentrations below the detection limit of this procedure (10 $\mu g \cdot g^{-1}$).

In the feeding trials, alevins that had been exposed to high levels of Al as embryos consumed significantly fewer daphnia than other test groups (Fig. 3). During the 5-h feeding period, the high-Al group (pH 5.0 plus 200 μg $Al \cdot L^{-1}$) captured and consumed only about 50% as many daphnia as the control group. All groups of alevins appeared to actively search for food; therefore, appetite did not appear to produce the observed differences.

Discussion

Exposure to elevated Al concentrations appears to be primarily responsible for the sublethal effects on size and development of alevins. Al toxicity to fish has been intensively studied in recent years (Schofield and Trojnar 1980; Baker and Schofield 1982; Brown 1983; Ingersoll 1986). Mortality from Al has been generally found in oversaturated solutions at pH 5.0 (Schofield and Trojnar 1980; Baker and Schoefield 1982), but LaZerte (1984) suggested that such oversaturated conditions rarely occur in situ, and Hutchinson et al. (1987) found that more realistic Al concentrations produce mortality only in combination with near-lethal H^+ concentrations ($pH \leq 4.6$). Our study shows that Al at sublethal levels of H^+ can produce significant anatomical (size, shape), physiological (ion accumulation), and behavioural (feeding) effects that can potentially reduce the survival of alevins.

Growth in length during the period of yolk absorption is an important determinant of later survival of free-swimming fish (Braum 1967). Large fish have greater swimming ability (Webb 1976), making them more effective at capturing food and avoiding predators (Blaxter 1969; Taylor and McPhail 1985). Exposure of embryos to sublethal levels of H^+ and Al may therefore limit survival of resulting alevins through the observed growth reductions.

Alevins exposed as embryos to a 5-d pulse of elevated H^+ and Al were not only shorter in length, but exhibited reduced utilization of yolk material. Similar results have been reported from chronic exposure to low pH (Peterson and Martin-Robichaud 1986) or to low pH with elevated Al (Baker and Schofield 1982). A large yolk sac decreases the swimming speed of alevins (Bams 1967; Thomas et al. 1969) and delays the rapid growth that accompanies transition to exogenous feeding (Twongo and MacCrimmon 1976; Heming 1982). These detrimental effects of residual yolk on swimming ability and growth appear to negate any potential advantage of yolk as a nutrient reserve for survival during periods of low food availability. Lake trout alevins are omnivorous, feeding on a variety of planktonic and benthic organisms, and can tolerate food deprivation for several weeks (Gunn and Keller 1981).

One might expect that slow growth and reduced yolk utilization in exposed fish would simply delay emergence from the substrate, rather than produce alevins that are poor swimmers because of their length and body shape. However, the timing of emergence does not appear to be affected by body size (Elliott 1984), and alevins have been observed to emerge with varying amounts of remaining yolk (Carey 1985). There have been few direct studies of pH and/or Al effects on emergence time, but the existing information suggests that pulse exposure of embryos to acidic runoff water would have little effect on the time of emergence. In a laboratory experiment with brook trout embryos and alevins, Trojnar (1977) found no consistent relationship between incubation pH and emergence time. In a field study, Gunn and Keller (1981) observed that lake trout embryos suffered high mortality in acidic water but emerged within the same time period as embryos from a circumneutral control.

Ionoregulation in embryos is unique because inorganic ions are obtained from both the surrounding water and attached yolk reserves. The relative importance of these two sources of ions varies with the particular element and the developmental state of the embryo (Peterson and Martin-Robichaud 1986). Mg^{2+} and Cl^- are obtained primarily from the yolk (Hayes et al. 1946), while Ca^{2+}, Na^+, and K^+ are obtained from both sources (Hayes et al. 1946; Natochin et al. 1976; Peterson and Martin-Robichaud 1986). In Atlantic salmon (*Salmo salar*) embryos, Peterson and Martin-Robichaud (1986) estimated that 70% of Na^+, 40% of K^+, and 85% of Ca^{2+} were derived from the ambient water. The latter cations increased in concentration in the body over time while declining in concentration in the yolk.

The reduced levels of whole-body ions in alevins, exposed as embryos to acid and Al, appear to be largely due to a delay in the rate of embryonic development, rather than the result of persistent ionoregulatory dysfunction. Only the intracellular electrolytes, Ca^{2+} and K^+, were significantly affected by the exposure conditions; the predominantly extracellular ions, Na^+ and Cl^-, which usually exhibit the most rapid and greatest whole-body losses during ionoregulatory failure (Dunson et al. 1977; Wood and McDonald 1982; Fraser and Harvey 1983; Lacroix et al. 1985), were much less or not affected. Some undetected efflux of electrolytes may have occurred during the exposure period; however, by the end of the recovery period, there were no significant net losses of any of the ions relative to initial control values.

The embryos exposed to Al had greater remaining yolk reserves, less whole-body Ca^{2+} and K^+, and delayed calcification of the skeleton, all of which are symptoms of delayed ontogeny. As embryos complete yolk absorption, they enter a period of rapid uptake of Ca^{2+} and K^+ from the water, a developmental period during which the mineralization of the skeleton is initiated (Hayes et al. 1946; Talbot et al. 1982). Peterson and Martin-Robichaud (1986) have suggested that exhaustion of some threshold level of Ca^{2+} in the yolk material may trigger active uptake from the water. Al appears to interfere with this process, but the mechanism producing the ontogenetic delays is unknown.

Little information exists to assess the ecological significance of the observed delays in skeletal development. Nelson (1982) found that chronic exposure of rainbow trout (*Salmo gairdneri*) embryos to low pH delayed calcification of jaw teeth and branchiostegal rays and suggested that these effects may reduce feeding efficiencies of alevins. Our results indicate that jaws and teeth are some of the first structures to calcify, indicating that condition of the teeth do not explain the poor performance of H^+- and Al-exposed fish in the feeding trial.

The results from the feeding trials suggest that pulse exposure of embryos to sublethal levels of H^+ and Al may reduce alevin survival in situ. Initiation of first feeding is a critical developmental step in fish and is often a period of high mortality in natural systems (Latta 1962; Elliott 1984). Onset of exogenous feeding appears to be highly sensitive to environmental stresses, including acidification (Farmer et al. 1980; Skogheim and Rosseland 1984; Lacroix et al. 1985). While loss of appetite or reduced food intake, during or shortly after exposure to acidic water (Lacroix et al. 1985; Lemily and Smith 1985; Cleveland et al. 1986), have been observed, latent effects after an extended recovery period have not been previously reported. The causal factor(s) for the poor performance of Al exposed fish is unknown. Effects on swimming stamina, agility, and sensory perception may all be involved, with the combined effect being of obvious disadvantage to the emerging alevin in nature.

These findings indicate that predictions dependent only on the occurrence of directly lethal chemical conditions may underestimate the detrimental effects of acidification on natural populations of lake trout. Sublethal effects, some of which are expressed well after the fish have been exposed to acidic runoff water, can extend the chemical range over which detrimental effects occur. The reduced growth and feeding efficiency observed in this experiment may limit the ability of alevins to compete with more tolerant species. These latent sublethal effects of pulse exposure to acidic, Al-rich water may help explain the disappearance of lake trout populations from lakes with relatively high ambient pH.

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Chemical Analysis of Muscle from Walleye (*Stizostedion vitreum vitreum*) with Myofibrogranuloma, a Chronic Myopathy[1]

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Kelly, R. K., J. F. Klaverkamp, R. V. Hunt, and O. Nielsen. 1987. Chemical analysis of muscle from walleye (*Stizostedion vitreum vitreum*) with myofibrogranuloma, a chronic myopathy. Can. J. Fish. Aquat. Sci. 44: 1425–1431.

Myofibrogranuloma (MFG), a chronic myopathy of unknown origin, is a rare disease of walleye (*Stizostedion vitreum vitreum*). We studied 15 cases observed in adult walleye during 1975–85 from seven lakes in southern Manitoba and northwestern Ontario and from one lake in North Dakota. Two of the lakes (Pelican Lake, Man., and Lake Sakakawea, ND) had a higher number of overt cases in 1984 and 1985. The inorganic analyses of representative samples of diseased muscle revealed that the ratio of potassium to sodium ions in the afflicted skeletal muscle was significantly depressed from the normal. Selenium was elevated. Calcium levels averaged 55 times normal in the muscle lesions where excessive calcium deposition was also readily demonstrable by histochemical staining with nuclear fast red. Magnesium levels were significantly depressed (i.e. 0.7 times normal) in diseased muscle. In contrast, copper (1.71 ± 0.57), iron (9.23 ± 4.56), zinc (25.48 ± 9.33), and aluminum (2.80 ± 1.14) concentrations (micrograms per gram wet wt ± SD) were generally 5- to 10-fold greater in the muscle lesions than in muscle from healthy walleye. The physiological significance of the latter data was unclear.

Le myofibrogranulome, une myopathie chronique d'origine inconnue, est une maladie rare du doré jaune (*Stizostedion vitreum vitreum*). De 1975 à 1985, les auteurs ont étudié 15 cas de maladie chez des dorés jaunes adultes provenant de sept lacs du sud du Manitoba et du nord-ouest de l'Ontario et d'un lac du Dakota du Nord. Dans deux des lacs (lac Pelican (Manitoba) et lac Sakakawea (Dakota du Nord)), ils ont observé un nombre plus élevé de cas évidents en 1984 et 1985. Les analyses inorganiques d'échantillons représentatifs de muscles touchés ont révélé que le rapport entre les ions de potassium et de sodium était nettement abaissé par rapport au taux normal tandis que la teneur en sélénium était élevée. En moyenne, les teneurs en calcium étaient 55 fois plus élevées que la teneur normale dans les lésions musculaires où l'excès de calcium était facilement identifiable par coloration histochimique des noyaux au rouge solide. Les teneurs en magnésium étaient nettement abaissées (0,7 fois la teneur normale) dans les muscles atteints. Par contre, les concentrations de cuivre (1,71 ± 0,57), de fer (9,23 ± 4,56), de zinc (25,48 ± 9,33) et d'aluminium (2,80 ± 1,14) (micrograms par gram poids humide ± EcT) étaient généralement de 5 à 10 fois plus élevées dans les lésions musculaires que dans les muscles de doré jaune en santé. La signification physiologique de ces dernières données n'est pas claire.

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Myofibrogranuloma (MFG) is a rare myopathy that has been described in adult walleye (*Stizostedion vitreum vitreum*) from scattered locations in the midwestern United States (Economon 1975; Mayes 1976; Holloway and Smith 1982). The geographic range of MGF has not been delineated, but the disease in Minnesota is generally more prevalent in fish from the more eutrophic lakes where the frequency of MFG approaches 1 in 10 000 walleye (Economon 1975, 1978). Natural mortality has never been recorded, and afflicted fish are normal externally even when severe internal damage to skeletal muscle exists.

Economon's (1975, 1978) thorough investigations on the pathology of the disease and its distribution in Minnesota were based upon 32 cases taken from 25 lakes and ponds from 1959 to 1974. Economon (1975, 1978) and Mayes (1976) were unable to find evidence that infectious agents were involved in MFG. Their careful investigations were limited primarily to the extensive use of selective staining of histological sections, mainly because fresh, nonfrozen tissues were generally unavailable for more sophisticated microbiological testing. Economon (1978) concluded that MFG was a muscular dystrophy-like anomaly and noted many similarities in the histopathology to that of progressive muscular dystrophy in humans. Holloway and Smith (1982) were more conservative in their description of the myopathy in a single specimen (from Wood Lake, ND) which appeared similar to those walleye described by Economon (1975, 1978).

From 1975 through 1985 we have confirmed 12 cases of MFG from lakes in Manitoba and northwestern Ontario and 3 cases from Lake Sakakawea, ND. The walleye were obtained from local fishermen by regional biologists and submitted to the Freshwater Institute for diagnosis. In every case the characteristic opaque, yellowish brown, rough-textured flesh of the

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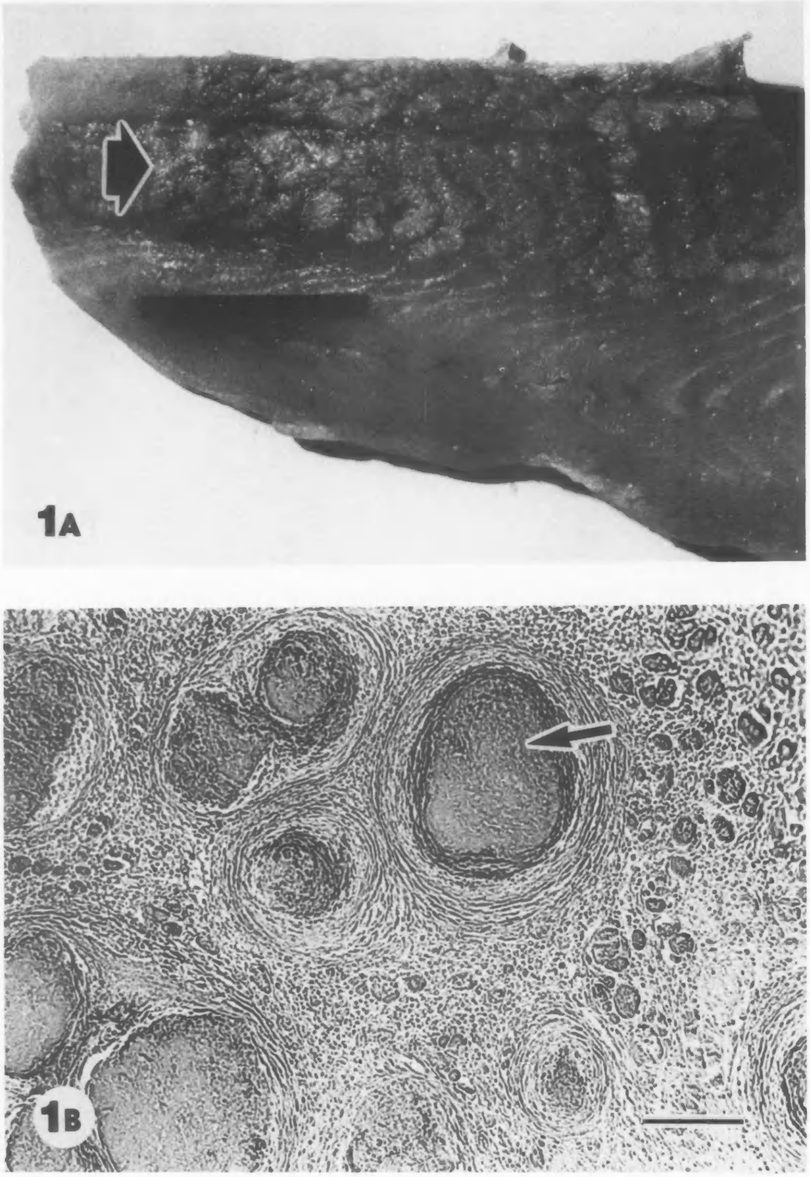

FIG. 1. (A) Myofibrogranuloma (MFG): gross pathology. Note the degenerative rough-textured, swollen musculature (arrow), typical of MFG. Specimen is from Pelican Lake, Man. Bar = 3.0 cm. (B) MFG: histochemical staining of transverse section of MFG-afflicted skeletal muscle showing abnormal (rounded) muscle fibers. Note wide variations in muscle fiber size and concentric cellular encirclements around typical pseudohypertrophic fiber (arrow). Sections are stained by the Calcium-Red Method of McGee-Russell (1955). Specimen is from Lake Sakakawea, ND. Bar = 0.1 mm.

diseased fish was a major cause of alarm to the fishermen (see Fig. 1A).

The purpose of the present report is to alert researchers to the occurrence of this myopathy of walleye in Canada and to provide information about significant chemical abnormalities associated with the diseased muscle.

Materials and Methods

Specimens

Because all of the diseased walleye and some of the normal ones were originally supplied on a nonseasonal basis by individual fishermen, we had little control over how specimens were handled prior to diagnosis. Some fish were supplied as fillets or as partially gutted carcasses. Consequently, age, sex, and size were not tabulated. The comparative adult control fish from Lake Winnipegosis and Dauphin Lake were caught by gill net and stored frozen for subsequent chemical analysis.

Histopathology

Fillets of skeletal muscle from MFG-afflicted fish were photographed using a Nikon Nikkormat 35-mm camera with a 55-mm close-up lens. Samples of normal and abnormal muscle were then excised and fixed in Bouin's solution or in 10% neutral buffered formalin. The fixed tissues were dehydrated, embedded, and sectioned by standard histological procedure (Humason 1979). The 10-μm tissue sections were stained with hematoxylin and eosin (H & E), or alternatively with nuclear fast red by application of the Calcium-Red Method of McGee-Russell (1955). The stained sections were examined with a Zeiss photomicroscope (see Fig. 1B). Material from the first field case obtained in 1976 was embedded and sent to the Fish Pathology Laboratory of the Minnesota Department of Natural Resources, St. Paul, MN 55155, where Philip Economon confirmed our initial diagnosis of MFG.

Chemistry

We analyzed skeletal muscle from MFG-afflicted walleye taken from three geographically distinct sites: Eagle Lake, Ont. (Fig. 2, site 5), Pelican Lake, Man. (Fig. 2, site 7), and Lake Sakakawea, ND (Fig. 2, site 8). Muscle was analyzed from control walleye caught in Lake Winnipegosis, Man. (Fig. 2, site 9), Dauphin Lake, Man. (Fig. 2, site 2), and Lake Sakakawea, ND. With the exception of Al, standard atomic absorption spectrophotometric procedures were used to determine metal and trace element concentrations in fish muscle.

For analysis of Na, K, Mg, Ca, Fe, Cu, Zn, Ni, and Al, from 4 to 6 g of muscle tissue was digested with nitric (5 mL) and perchloric (2 mL) acids and made up to 25 mL with deionized water. After the addition of CsCl solution as ionization buffer to a subsample of the digest (final concentration 1000 μg Cs/mL), Al was determined by direct current plasma emission spectrometry (Spectraspan IIIB). The remaining elements were analyzed by flame atomic absorption spectrophotometry (Varian AA5). (For Cd, only a limited number of analyses were done, and these were based upon only 1 g of sample.)

For analysis of Se and As, wet muscle tissue (0.5 g) was analyzed by the method of Vijan and Wood (1976). Wet tissue (0.2 g) was analyzed for Hg by the method of Armstrong and Uthe (1971). The detection limits (micrograms per gram) for trace elements were as follows:

Fe	Cu	Zn	Al	Ni	Cd	As	Se	Hg
0.1	0.05	0.02	0.1	0.1	0.2	0.05	0.05	0.01

Limits were based on a sample weight of 5 g (wet wt) except for Cd (1 g), Se and As (0.5 g), and Hg (0.2 g).

Data Analysis

Unless indicated, the chemical analyses for metals and trace elements were conducted on MFG-afflicted skeletal muscle from 10 walleye (4 from Lake Sakakawea, 5 from Pelican Lake, and 1 from Eagle Lake). Muscle that appeared normal by gross visual inspection was also taken from 8 of these same 10 diseased fish and was analyzed for metals and trace elements. Control analyses were done on skeletal muscle from 20 apparently healthy, normal walleye without MFG (10 from Lake Winnipegosis, 8 from Dauphin Lake, and 2 from Lake Sakakawea). Thus, irrespective of sampling site, the walleye muscle samples used for the elemental analyses are subsequently grouped as follows: (1) myofibrogranuloma (MFG), (2) normal appearance (NA), and (3) normal.

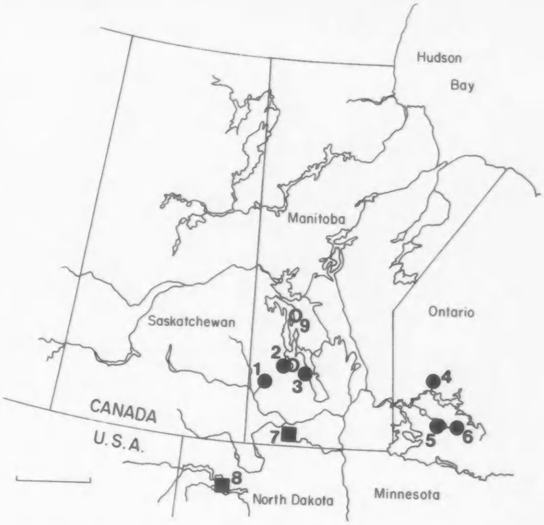

FIG. 2. Source of MFG cases. ●, individual samples submitted by fisherman to regional biologists (1975–85); ■, multiple samples submitted by fishermen to regional biologists (1984–85); ○, normal control walleye. Bar = 220 km.

Analytical data within each major group from each lake were expressed as $\overline{X} \pm$ SD and analyzed for significant differences ($P \leq 0.05$) by Student's *t* test. While strict adherence to testing for homogeneity of variance was not considered necessary for evaluating differences in the data, it was readily apparent from the large variances that the Ca concentration data were not homogeneous. Therefore, these data were log transformed. If differences among lakes within a group were not significant ($P \leq 0.05$), the data were combined for that group (viz. Ca concentrations in all three groups, Fig. 3A). If any differences within a group were significantly different (viz. Mg concentrations in normal fish, Fig. 3B), the data were not combined. In cases where data are combined, differences between MFG and normal samples were also analyzed by using Student's *t* test. In cases where data could not be combined, these differences were analyzed by comparing all separate values of the means and standard deviations. Differences were considered real if all separate MFG values ($\overline{X} \pm$ SD) were statistically significant ($P \leq 0.05$) from all normal values ($\overline{X} \pm$ SD). Differences between metal and trace element data from NA and normal samples were analyzed using the same approach.

Results

Over an 11-yr period, walleye with characteristic MFG (Fig. 1A) were taken on a nonseasonal basis from a number of diverse lakes in Manitoba, northwestern Ontario, and North

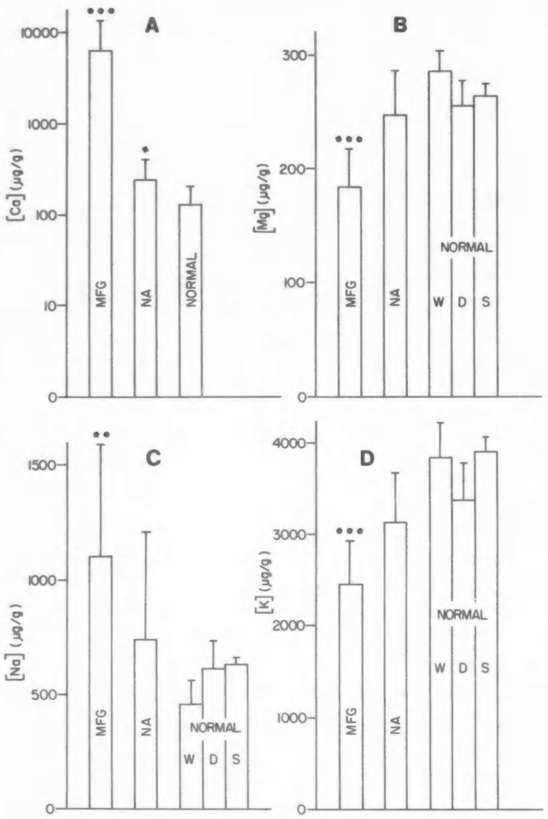

FIG. 3. Concentrations of Ca, Mg, Na, and K in walleye muscle (diseased (MFG), normal appearance (NA), healthy (normal)). Data are expressed as $\bar{X}$ ± SD µg/g on a wet weight basis. Data from different lakes were combined when no significant differences existed. For normal samples, W = Lake Winnipegosis (n = 10), D = Dauphin Lake (n = 8), and S = Lake Sakakewea (n = 2). Significant differences from normal values: $*P \leq 0.05$, $**P \leq 0.005$, and $***P \leq 0.0005$.

Dakota (Fig. 2). Internal organs were missing from many of the 15 specimens sent to out laboratory; however, both males and females were represented. The extremely low prevalence of lesions and the absence of any externally visible indicators of the myopathy precluded the use of freshly caught specimens for biochemical analyses or for ultrastructural studies. Gross lesions were readily apparent in fillets and were distinguishable from normal tissue by the yellowish appearance of the rough-textured degenerative flesh (Fig. 1A) which typically appeared in the lateral trunk musculature often in areas adjacent to the spinal column, as described by Economon (1978). The atrophic and pseudohypertrophic muscle fibers, which are pathognomonic for the disease (Economon 1975, 1978), were readily visible in histological sections observed with low-power light microscopy (Fig. 1B). Because we lacked an accessible source of fresh disease material and were also unable to obtain any walleye with early-stage lesions, a more detailed description of observed cellular changes and infiltrative cell types was not attempted.

Substantial amounts of Ca were present in tissue sections of MFG-afflicted muscle which were readily stained with nuclear fast red, a Ca-specific histochemical stain (Fig. 1B). Muscle tissues from control fish did not stain, but remained colorless. The mean level of Ca in MFG-diseased muscle was 55 times greater than that in muscle from healthy fish (Fig. 3A). Muscle samples having a normal appearance (NA) from diseased fish also had significantly higher Ca levels than samples of muscle from healthy fish (Fig. 3A), but the concentrations were less than 4% of those found in diseased muscles.

Imbalances in Mg and the alkali metals Na and K, which are essential for normal physiological performance of skeletal muscle, were found in samples of diseased walleye muscle (Fig. 3B, 3C, and 3D). Average Mg levels in MFG-affected muscle ranged from 64% of the concentration found in Lake Winnipegosis fish to 72% of the level for Dauphin Lake walleye (Fig. 3B). In MFG muscle, mean Na levels were 2.4, 1.8, and 1.8 times those found in healthy fish from Lake Winnipegosis, Dauphin Lake, and Lake Sakakawea, respectively (Fig. 3C). Average concentrations of K in MFG muscle were 64, 73, and 63% of the levels found in walleye muscle from these respective lakes. Concentrations of Mg and K in NA muscle (Fig. 3B and 3D) were not significantly different from Dauphin Lake normal samples, but were lower (P = 0.01) than levels found in normal Lake Winnipegosis walleye. Sodium concentrations in NA muscle (Fig. 3C) were not significantly different from levels found in samples from healthy fish.

In diseased muscle, average Al, Cu, and Zn levels were 5.5, 6.6, and 7.1 times higher, respectively, than those concentrations found in muscle from healthy fish (Fig. 4). Combined Fe levels in diseased muscle were 2.5, 5.1, and 10.1 times higher than in healthy walleye muscle from Lake Sakakawea, Dauphin Lake, and Lake Winnipegosis, respectively. Concentrations of Ni were below detection limits (0.1 µg/g) in all samples from healthy fish and in seven of the eight NA samples from diseased fish. One of the NA muscles contained 0.45 µg Ni/g. In MFG-diseased muscle, Ni was below detection limit in one sample from Pelican Lake, but was present at 0.6 ± 0.5 µg Ni/g in the remaining nine samples. Analyses for As and Cd were conducted on three MFG samples, two NA samples, and five samples from healthy fish, and all concentrations were below limits of detection. No significant differences in Hg concentrations were observed between MFG, NA, and normal groups in 18 samples (5 MFG, 3 NA, and 10 normal). The Hg levels in skeletal muscle appeared to depend on the lake of origin rather than the disease state of the tissue.

Diseased muscle from fish in Lake Sakakawea and Pelican Lake contained significantly higher Se concentrations than those levels found in healthy fish from Lake Winnipegosis, Dauphin Lake, and Lake Sakakawea (Fig. 5). The differences ranged from 1.4-fold between Pelican Lake (MFG-diseased) and Dauphin Lake samples to 9.2-fold between Lake Sakakawea (MFG-diseased) and Lake Winnipegosis samples. Because Se is known to affect concentrations of metals in Groups IB and IIB in vertebrate tissues, linear regression analyses were performed between molar concentrations of Se and those of Zn, Cu, and Zn plus Cu. No significant correlations were observed.

The water content was 78.4 ± 1.6% (n = 18), 78.8 ± 1.0% (n = 6), and 73.9 ± 3.6% (n = 8) in normal, NA, and MFG muscle samples, respectively. While the water content of MFG-diseased muscle was significantly lower than that of normal and NA muscle, the differences do not account for the

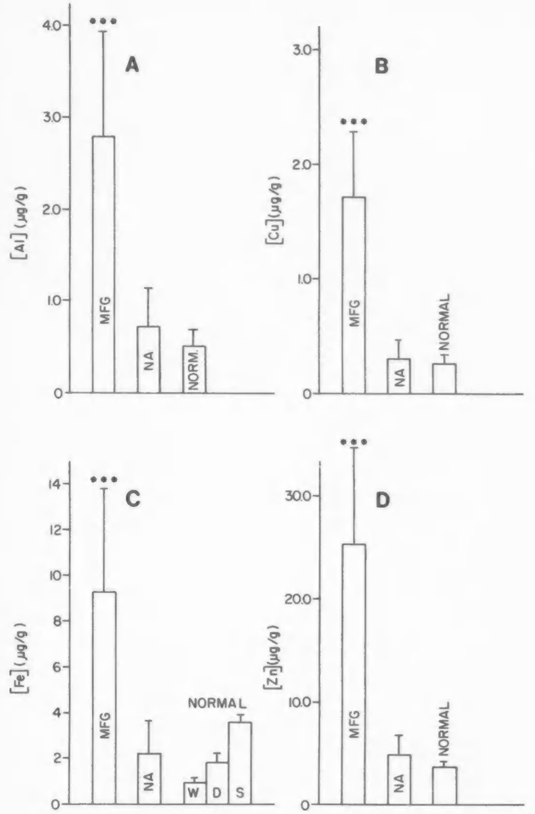

FIG. 4. Concentrations of Al, Cu, Fe, and Zn in MFG, NA, and normal muscle of walleye. Abbreviations, expression of data, statistical significance of differences, and numbers of analyses are the same as in Fig. 3.

elevated concentrations of Ca, Na, Al, Cu, Fe, Zn, and Se. Furthermore, the close agreement between the hydration of the controls (78.4%) and the diseased fish (78.8% for the nonlesion material) indicates that variation in handling between diseased specimens and controls is not a factor in the comparative elemental analyses.

Discussion

These results confirm that MFG in walleye is widely dispersed geographically and occurs in both the Hudson Bay drainage and the Missouri River watershed. Economon (1975, 1978) reported that MFG was most common in walleye from small stocked eutrophic lakes, in Minnesota, but that the prevalence of grossly afflicted mature walleye from such lakes was likely not significantly greater than 0.01%. In 1983 and 1984 the frequency of MFG in walleye from Lake Sakakawea was many fold higher than this level (Mr. Fred Ryckman, North Dakota Game and Fish Department, Bismarck, ND 58505, pers. comm.). Myofibrogranuloma was also recently found to occur at higher prevalence in walleye from Fife Lake, Saskatchewan (Dr. Joan Bernstein, Department of Applied Microbiology and Food Science, University of Saskatchewan, Saskatoon, Sask. S7N 0W0, pers. comm.). A similar localized increase may have occurred in Pelican Lake in Manitoba in 1984. Unfortunately, Pelican Lake experienced an extensive winter kill early in 1985 shortly after the fish samples were collected, and accurate disease prevalence data were not obtained. Among the lakes in our present study, both Lake Sakakawea and Pelican Lake are suitable sites for further investigations of the disease etiology and the potential role of contributing environmental factors of the type reviewed by Gilbertson (1984).

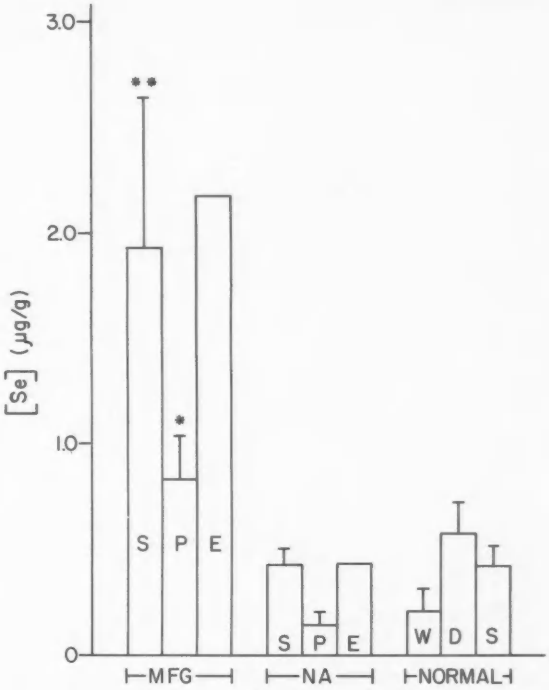

FIG. 5. Concentrations of Se in MFG, NA, and normal muscle of walleye. Abbreviations, expression of data, statistical significance of differences, and numbers of analyses as in Fig. 3. For MFG and NA samples, S = Lake Sakakawea (n = 4 MFG and 4 NA), P = Pelican Lake (n = 5 MFG and 3 NA), and E = Eagle Lake (n = 1 MFG and 1 NA).

Comparing elemental analytical data presented in this study with similar data from other studies on walleye or other freshwater fish species is extremely difficult because few detailed studies have been published. Most investigations of freshwater fish focus on one of three aspects: (1) the physiological actions of essential major alkali and alkaline earth elements of Na, K, Ca, and Mg, (2) the catalytic co-factor roles of essential trace elements such as Cu, Fe, Zn, and Se, or (3) the adverse effects of pollutant elements such as Al, As, Cd, Hg, Ni, and Pb (George 1982). These investigations, however, have not used walleye as an experimental animal (Love 1980; Colby et al. 1979).

Concentrations of essential elements found in skeletal muscle from healthy walleye in this study are similar to those found in rainbow trout (*Salmo gairdneri*) (Shearer 1984). A survey of Cu, Se, and Zn concentrations (micrograms per gram wet weight) in muscle from 152 white suckers (*Catostomus com-*

mersoni) and 142 northern pike (*Esox lucius*) from 13 lakes in northern Manitoba and Saskatchewan demonstrated that Cu concentrations ranged from 0.24 to 0.31 in white suckers and 0.12 to 0.21 in northern pike; Se values were 0.23 to 1.03 in suckers and 0.23 to 0.87 in pike; and Zn concentrations ranged from 3.0 to 5.2 in suckers and 4.4 to 5.7 in pike (J. F. Klaverkamp, unpubl. obs.). These values are also close to concentrations observed in skeletal muscle from normal walleye or rainbow trout (Shearer 1984).

In MFG-diseased muscle, the pronounced alterations in Ca, Mg, Na, and K concentrations would likely be associated with severely reduced muscle function. In the series of 32 MFG-diseased walleye collected by Economon (1978) over a period of 15 yr, only 2 fish exhibited symptoms of paralysis when captured. Economon (1978) also reported that mineralization or calcification was apparent in histological sections only from fish in advanced stages of degeneration. Consequently, MFG-affected muscles analyzed in our study may have been from fish in the later stages of the disease.

Histological features of MFG in walleye have common and distinguishing characteristics similar to genetically acquired dystrophic muscle processes occurring in domestic species of birds and mammals (Economon 1978). Muscle biopsies from humans having progressive muscular dystrophy or certain related myopathies also have excessive Ca accumulation (Oberc and Engel 1977; Bodensteiner and Engel 1978) associated with a marked reduction in Mg (Bertorini et al. 1982). Potassium ions (K^+) are actively accumulated across functional cellular membranes whereas Ca^{2+} has an essential cytoplasmic role in the physiological regulation of muscle contraction. Thus, the observed low K to Na ratio and the excessive accumulation of Ca with depression of Mg is consistent with extensive damage to the muscle membrane systems. Wrogemann and Pena (1976) have suggested that a common mechanism exists for many myopathies and that a defect or dysfunction in muscle fiber plasmalemma results in a net inflow and intracellular accumulation of Ca, followed by progressive cellular damage. Whether there is a primary or a secondary defect in membrane function in MFG is not yet understood. Unfortunately there are presently no ultrastructural data for MFG-afflicted skeletal muscle.

Molecular mechanisms involved in the repair of damaged or defective membranes could explain the elevated concentrations of the catalytic co-factors Se, Cu, Zn, and Fe found in the MFG-afflicted skeletal muscle (Fig. 4 and 5). For example, Se, Cu, and Zn are involved in terminating cellular and subcellular damage induced by lipid peroxidation of membranes which is a pathologic process common to adverse effects produced by mechanical injury, pathogenic microorganisms, radiation, and toxic chemicals (Sies and Cadenas 1983; Cerutti 1985). Glutathione peroxidase terminates the damage produced by intracellular hydroxyl free radicals and requires Se as a co-factor (Wendel 1980). Cu and Zn are co-factors of the enzyme superoxide dismutase which eliminates reactive superoxide free radicals (Hassan and Fridovich 1980; Fridovich 1983). Cu, Fe, and Zn are co-factors of other enzymes and are transported by nonenzymatic metal-binding proteins (George 1982). Additional research on these essential metals in freshly collected MFG-affected muscle is required to understand the role of these elements and possibly to develop diagnostic tools useful for detecting early stages of the disease.

It is difficult to interpret the elevated Al concentrations in diseased muscle. The complexation and bioavailability of waterborne Al is highly dependent on pH, fluoride, and dissolved organic carbon (LaZerte 1984; Jones and Bennett 1986). The lakes in this study ranged from mesotrophic to eutrophic, and therefore, most of the Al would exist as complexes. The accumulation and distribution of Al by walleye through their food webs, as well as the Al content of food organisms in the lakes under study, are all unknown. Whether Al concentrations are significantly elevated in vertebrate myopathies, other than MFG, has not been determined.

Progressive muscular dystrophy in humans is a genetic disorder that is generally sex linked (Mrak 1985). In the case of Duchenne dystrophy, the defect occurs in a defined region of the X chromosome (Kunkel et al. 1986). Similar chronic diseases occur in animals and may either be genetic in origin or may be caused by nutritional factors. The latter type of myopathies have commonly been reported in many domesticated avian and mammalian species and are usually due to a dietary deficiency of vitamin E (α-tocopherol) or of Se (Hadlow 1973). Identical dietary-induced myopathies probably occur in cultured fish (Mawdesley-Thomas 1975; Miyazaki 1986). Economon (1978) postulated that MFG might be a genetic disorder of walleye, but the disease is apparently not sex linked. Whether or not dietary factors are important in the pathophysiology of this disease is unknown. Although the Se levels were significantly above normal in the skeletal muscle lesions (Fig. 5), the biological availability of this trace element to the living fish is uncertain. The disease in walleye likely represents a unique myopathy of wild fish.

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Empirical Models of Fish Response to Lake Acidification

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A large historical data set from the Adirondack region of New York was compiled to study the relationship between water chemistry variables associated with acid precipitation and the presence/absence of selected fish species. The data set was used to examine simple statistical models for fish presence/absence, as a function of the water chemistry variables, for brook trout (*Salvelinus fontinalis*), lake trout (*Salvelinus namaycush*), white sucker (*Catostomus commersoni*), and yellow perch (*Perca flavescens*). Of these models, only those for brook trout and lake trout were found to be acceptable based on statistical goodness-of-fit criteria; thus, parameters for models of these two species alone were estimated using maximum likelihood logistic regression. Candidate models for brook trout and lake trout were then examined, with particular consideration for the problems associated with model misspecification, errors-in-variables, and multicollinearity. For each of the two species, a model was recommended that may be used to predict the effect of changes in lake acidification on species presence/absence in lakes in the Adirondack region.

Une importante série de données historiques recueillies dans la région de l'Adirondack (État de New York) a été rassemblée afin d'étudier la relation entre les variables chimiques de l'eau liées aux précipitations acides et la présence ou l'absence de certaines espèces de poissons. La série de données a servi à l'examen de simples modèles statistiques sur la présence ou l'absence de poissons en fonction des variables chimiques de l'eau, plus particulièrement de l'omble de fontaine (*Salvelinus fontinalis*), du touladi (*Salvelinus namaycush*), du meunier noir (*Catostomus commersoni*) et de la perchaude (*Perca flavescens*). De ces modèles, seuls ceux traitant de l'omble de fontaine et du touladi se sont révélés acceptables d'après certains critères statistiques sur la validité de l'ajustement. Ainsi, seuls les paramètres utilisés dans l'élaboration des modèles pour ces deux espèces ont été évalués à l'aide d'une régression logistique de la vraisemblance maximum. On a ensuite examiné les autres modèles élaborés pour l'omble de fontaine et le touladi en portant une attention particulière aux problèmes associés au défaut de sensibilité du modèle, aux erreurs de variables et à la multicolinéarité. Pour ces deux espèces, on recommande un modèle qui peut servir à prédire l'incidence de variations de l'acidification lacustre sur leur présence ou leur absence dans des lacs de la région de l'Adirondack.

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Both experimental and observational studies provide scientific support for policy decisions concerning acid deposition. Experimental evidence suggests that acid precipitation is a likely cause of the absence of fish populations in selected North American lakes (Baker 1985). Observational evidence (Baker and Harvey 1984; Muniz et al. 1984; Beggs et al. 1985) supports this experimental information with inferences based on the study of cross-sectional, or regional, data sets. Observational information of this type is expected to be particularly useful for policy evaluation, since the regional nature of lake acidification is likely to mean that policy assessment will be regional in nature as well.

To provide an observational basis for policy evaluation, a large cross-sectional data set was examined for the purpose of developing models of fish response to lake acidification. The data set, FIN (Fish Information Network), was assembled by Baker and Harvey (1984) from available historical information on water chemistry and fish surveys for lakes in the Adirondack region of New York State. These data were acquired from studies undertaken by a variety of investigators for a variety of purposes using a variety of analytical methods. Baker and Harvey (1984) described the data set and provided many useful background statistical investigations of the data.

The objective of the work presented here is the development of statistical models of fish response to lake acidification for use in regional policy assessment. Since the model parameters are affected by the method of estimation used, and since the models reflect patterns in the historical data set, attention is focused on the selection of an appropriate statistical method for estimation and on the effect of characteristics of the data set (e.g. errors of observation) on evaluation and interpretation of the models. Based on data availability and/or expected sensitivity to acid precipitation, the following four fish species were selected for examination in this study: brook trout (*Salvelinus fontinalis*), lake trout (*Salvelinus namaycush*), white sucker (*Catostomus commersoni*), and yellow perch (*Perca flavescens*). The primary chemistry variables considered were pH, calcium (Ca), alkalinity (Alk), and total aluminum (Al), all of which have been found to be important in experimental and observational studies of fish survival (Baker 1985).

The paper is organized as follows. First, methods that may be used to estimate parameters for the presence/absence models are discussed. The objectives of this section are to provide the basis for selection of maximum likelihood logistic regression and to consider problems with the data set that may affect estimation and statistical inference. The next section presents the models of presence/absence for the fish species. This includes a discussion of the existing literature on fish response models, descriptive statistics for the four species subsets of the FIN data set, and a presentation of candidate models. The last major section presents the principal goodness-of-fit criterion employed here, classification error, and a discussion of the candidate models that concludes with a recommendation on preferred models for brook trout and lake trout.

Methods of Parameter Estimation

Three methods of parameter estimation were considered for selected purposes in the development and evaluation of empirical models of fish response. These methods are ordinary least squares regression analysis, maximum likelihood logistic regression analysis, and linear discriminant analysis. In situations where the dependent variable is continuous and the model is specified as linear in the parameters, linear regression analysis using ordinary least squares is generally the preferred method of parameter estimation. This classical regression model does not hold, however, when the dependent variable is dichotomous rather than continuous because of failure of the homoscedastic error assumption. This situation arises, for example, if the dependent variable assumes the value 0 or 1, associated with fish species absence or presence. Even with this assumption violation, linear regression may still be useful in the exploratory stage of a dichotomous dependent variable model (Amemiya 1981), and it also may be used to directly estimate parameters for the two-group discriminant analysis (Flury and Riedwyl 1985) considered below.

Regression analysis with a dichotomous dependent variable (Maddala 1983) is typically expressed in terms of a logit or probit model. The probit model, based on the cumulative normal distribution function, is less easily parameterized than is the logit (but otherwise is quite similar), so it is not considered here. The logit, or logistic, model is the cumulative logistic probability distribution function

$$(1)\quad \Pr(P)_i = \frac{1}{1 + \exp - (\beta_0 + \sum \beta_k x_{ik})}$$

where x_{ik} are observable independent variables, β_0 and β_k are unobservable parameters (β_0 is an intercept term), and $\Pr(P)_i$ is the probability that case i has the fish species present. The logistic model has domain 0−1, which leads to the direct interpretation of the response variable as a probability. The logistic probability density function is similar in shape to a normal distribution. In fact, when the probit (normal) and logistic models are fit to the same data set and scaled for comparison, the probit model is a t-distribution with infinite degrees of freedom, while the logistic model resembles a t-distribution with 7 degrees of freedom (Hanushek and Jackson 1977), or in other words, the logistic is approximately a normal distribution with fatter tails.

The parameters of the logistic model can be estimated using maximum likelihood logistic regression; details of the estimation procedure are given in Pindyck and Rubinfeld (1981). The assumption inherent in this procedure is that the probability of an event (fish species presence or absence), conditional on the independent variables (x_k), is correctly specified by Equation 1. Maximum likelihood estimation of the model parameters results in consistent and asymptotically (large sample) efficient and normal estimators (Pindyck and Rubinfeld 1981). Thus, t-statistics can be applied to test the significance of individual parameters.

Another frequently used procedure for a model with a dichotomous dependent variable is discriminant analysis (Green 1978). For a two-group (dichotomous) dependent variable, with equal independent variable covariance matrices within each of the groups, a linear discriminant function may be estimated by maximizing the ratio of the between-groups variation to the within-groups variation. A vector of weights results, and these weights are the parameters in the discriminant function. If it is assumed that the predictor variables are distributed within each group according to a known multivariate normal distribution and the groups have equal covariance matrices, then Bayes Theorem may be used to estimate the posterior probability of group classification with the logistic model. This is of interest because it indicates that discriminant analysis can yield classification probabilities in a manner analogous to logistic regression.

Of all the methods of parameter estimation considered, maximum likelihood logistic regression is preferred for the determination of final parameter values. Previously, linear regression analysis was rejected due to lack of homoscedasticity in the error term, and the probit model was rejected because of computational difficulty (relative to the logistic model). Maximum likelihood logistic regression is generally preferred over discriminant analysis because the assumptions (equal covariances and normality) required to achieve desirable properties of the linear discriminant function probably rarely hold in practical, multivariate situations and are extremely difficult to confirm. Previous studies (e.g. Efron 1975; Press and Wilson 1978) have shown that where distributions are non-normal or unknown, maximum likelihood logistic regression is the robust option and should be preferred.

In concluding this section on methods of parameter estimation, it is useful to examine some problems related to the data and to scientific knowledge that will affect inferences from models based on the parameter estimates. Given the complexity of lake ecosystems, it is certain that the models are misspecified. A correctly specified model is one in which the mathematical expression (including the random error term) is the true description of reality. Model misspecification can therefore involve omission of a relevant variable, inclusion of an irrelevant variable, use of an incorrect functional form, or improper expression of the error term.

Correlation between or among exogenous variables, or multicollinearity, is another problem. When multicollinearity is present and the model is incorrectly specified (e.g. an important independent variable is omitted), parameter estimates will be biased. This can be particularly troublesome when a procedure like stepwise regression is used to develop candidate models, since inclusion or exclusion of a variable at each step can change the parameter estimates substantially. When multicollinearity exists and the regression model is correctly specified, the parameter estimates are unbiased. However, the variance will be inflated for all of the parameter estimates associated wth the x-variables involved in the multicollinearity. Parameters are less likely to be statistically significant and they may vary substantially from sample to sample. Since the water chemistry predictor variables are highly correlated, multicollinearity is of concern in this study.

A consequence of multicollinearity that is apparent in the fish response models presented below is that the absolute values of the estimated model parameters tend to be larger than the absolute values of the true (but unknown) model parameters in ordinary least squares regression. To be specific, if $\boldsymbol{\beta}$ is the vector of true regression parameters and $\hat{\boldsymbol{\beta}}$ is the vector of estimated parameters, the relationship between the expected squared lengths of these two vectors is (Draper and Van Nostrand 1979), for linear regression,

$$(2) \quad E(\hat{\boldsymbol{\beta}}'\hat{\boldsymbol{\beta}}) = \boldsymbol{\beta}'\boldsymbol{\beta} + \sigma^2 \sum (1/\lambda_i)$$

where E is the expectation operator, σ^2 is the error variance, and λ_i is the ith eigenvalue of the correlation matrix of the x-variables. For maximum likelihood logistic regression, Equation 2 is not exact, but it does indicate the effect of small eigenvalues on parameter estimates under multicollinearity. The eigenvalues (or the ratio of highest to lowest eigenvalues) are known to be good indicators of the presence of multicollinearity (Judge et al. 1985), as multicollinearity is typically associated with one or more extremely low eigenvalues. In that situation, the seond term on the right side of Equation 2 becomes large, and the squared (or absolute) length of the estimated parameter vector becomes too large relative to the true parameter vector. This effect is demonstrated, and its consequences are discussed, in the next section.

Among the possible solutions to multicollinearity that may address the effect presented in Equation 2 are shrinkage estimators such as ridge regression (Draper and Van Nostrand 1979) and principal components regression (Judge et al. 1985). In particular, principal components regression is appealing here, since the water chemistry variables are highly correlated with each other. This high correlation will result in a first principal component, reflecting the common acidity basis of the water chemistry variables, that should explain much of the variance in the independent variables. Accordingly, a logistic regression model was developed using the first principal component as the independent variable.

Yet another problem becomes apparent when one considers that most limnological data are measured with error or reflect natural variability. A consequence is that the observational error in an independent variable causes dependence between the regression error term and the independent variable. In a simple least squares regression model, this results in bias in the regression parameter; effects in multiple regression models are not so straightforwardly defined but may be calculated. For the fish response data, error can also occur in the presence/absence observations. Errors in both the dependent and independent variables were estimated (Reckhow et al. 1985), and a portion of that analysis is presented in a later section. Both dependent and independent varible error can affect predictive classification error in the application of a presence/absence fish response model. For example, Michalek and Tripathi (1980) showed that, with normally distributed independent variables, the logistic regression model can be expected to result in a lower classification error than the linear discriminant function when either of the above-mentioned errors is present.

Given the consequences of these problems on the model parameters, it is inappropriate to infer cause−effect from the "significance" of estimated parameters in these empirical fish response models. It is reasonable to demand models that are not inconsistent with theory; however, it is unreasonable to adopt a new theory to explain particular statistically significant parameter estimates for a logistic fish response model. The models are simply summarizing data associations, and it is likely that there are conditions other than cause−effect that determine parameter estimates and associated error terms.

Models of Fish Presence/Absence

Previous studies involving cross-sectional, or regional, empirical analyses of fish response to lake acidification provide useful background information and confirm the many experimental studies demonstrating the importance of pH, aluminum, calcium, and alkalinity in fish survival.

In Ontario, Beggs et al. (1985) used empirical models of lake trout presence/absence in a study that was similar in intent to that presented here. One hundred and seventy-three lake trout lakes, from an area known to receive high levels of acid deposition, were part of a water chemistry and fish netting survey of over 1200 lake trout lakes. The water chemistry and fish presence/absence data from the 173 lake subset were used to parameterize stepwise, and prespecified, discriminant functions. As Beggs et al. (1985) noted, conclusions concerning the importance and effect of predictor variables in their discriminant functions should not be based on these results because of problems like those identified in the last section. However, it may be observed that Beggs et al. achieved high apparent presence/absence classification success rates (95%+), based on predicted classification for the subset and complete data set, with various discriminant functions that included pH, aluminum, calcium, and alkalinity. Measures of fit of a presence/absence model, such as classification error, constitute an important topic in empirical model development and are discussed in the next section.

Another major regional data set for fish and water chemistry was published by Wright and Snekvik (1977) for Norwegian lakes. Brown and Sadler (1981) used these data to create separate pH histograms for lakes with "good" fish populations, "sparse" fish populations, and "no fish." From these histograms, they estimated the effect on fishery status of a 0.2-unit increase in pH. Muniz et al. (1984) revised and augmented the Wright and Snekvik data, based on a study by Sevaldrud and Muniz (1980). They examined pH histograms for lakes with "lost" and "good" brown trout (*Salmo trutta*) populations, for different conductivity classes. Like Brown and Sadler, Muniz et al. (1984) estimated the effect on fishery status of a 0.2-unit increase in pH. The substantal difference between the predictions from these two studies (9 and 21% of the lakes, respectively, are predicted to change fishery status) is indicative of the effect of data quality and methods of analysis on scientific inferences.

For the study described in this paper, statistical, ecological, and policy considerations resulted in certain constraints imposed on the data set for the purpose of empirical model development. Thus, analyses reported here concern mean values, for June−September data, taken at 0−1 m depth, in lakes not recently stocked (within 5 yr of water chemistry data used), and for which fish species presence was confirmed at some point in the past. For each fish species, only gillnet survey data were used. The lake chemistry data used were confined to a single analytical method. These constraints reduced the size of the data set but helped to eliminate nuisance variation.

Data analysis and empirical model specification were undertaken for brook trout, lake trout, white sucker, and yellow perch. For the independent variables, model specification strategy included examination of descriptive statistics and univariate distributions (to indicate appropriate transformations), examination of box and whisker plots (to indicate effective presence/absence discrimination variables), examination of bivariate presence/absence plots (to help visually identify bivariate discrimination patterns), and stepwise variable selection (generally using 0.05 as the cutoff significance level) for the logistic regression model. Following modeling strategies successfully employed in epidemiological studies (Kleinbaum et al. 1982), we created interaction (cross-product) terms from the water chemistry variables. If an interaction term is significant and thus included in the model, then both single-variable terms must also be included in the model. This is based on the hierarchy principle (Bishop et al. 1975) which derives from the fact that higher order (interaction) terms can be expressed as deviations from lower order (single-variable) terms. Use of interaction terms is not based on a priori knowledge of interaction, but rather is intended to provide additional terms that may be important based on statistical measures of fit. Interaction terms do exacerbate the multicollinearity problem; however, in at least one case they also substantially improve model fit. It must be emphasized that these models have a theoretical basis in the independent (water chemistry) variables selected; however, the actual model specification is empirical and is based on statistical goodness of fit.

As stated above, development of each logistic regression model was preceded by an analysis of the relevant data on the independent variables pH, calcium, aluminum, and alkalinity. For each species, the relevant data consisted of the mean values for these water chemistry variables in each of the lakes in FIN with gillnet survey results for that species. All concentration variables (and surface area) were log-transformed, since the marginal distributions of these variables were approximately lognormal. Base *e* was selected because it resulted in model parameters comparable in magnitude with the pH parameter; this is important for proposed Bayesian analyses based on an exchangeability assumption. Sample size was not constant across variables, reflecting the incomplete nature of the historical FIN data.

The box and whisker plots (e.g. Fig. 1) used to examine the independent variables require a brief explanation (see Reckhow (1980) for more details). A box and whisker plot is created from order statistics for a particular sample. The plot displays the maximum value in the sample, the minimum value, the median value (fiftieth percentile), and the values at the quartiles (the twenty-fifth and seventy-fifth percentiles). These five-order statistics are represented by the five solid and broken horizontal lines in each plot (see Fig. 1). The box portion of the plot is bounded above and below by the values at the quartiles. The width of the box is scaled by sample size; thus, if there are two box and whisker plots on a graph and the sample size of the first is twice that of the second, then the first box will be twice as wide as the second. Finally, the notch in the box around the broken line for the median is an approximate vertical scale for comparing medians among boxes at the 0.05 level. Thus, if the notches for two boxes do not overlap vertically, then the medians can be considered as significantly different at approximately the 0.05 level. Occasionally, the notch will be larger than the distance between the median and either quartile or extreme value, causing the plot to assume an unusual shape (see Fig. 3).

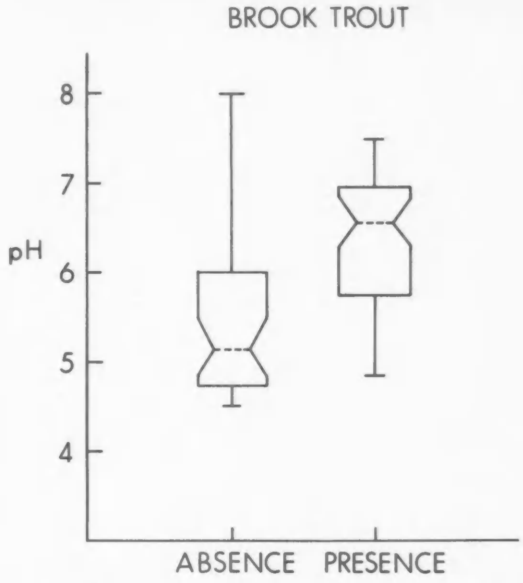

FIG. 1. pH box and whisker plots for lakes with brook trout "absent" and "present."

To begin the development of the first of the proposed models for the four fish species, we examined FIN data on brook trout lakes and found that the mean "absence" lake has lower pH, lower calcium, higher aluminum, lower alkalinity, and less surface area than does the mean "presence" lake (Table 1). These are all consistent with theory. The best predictor variable of the four chemistry variables appears to be pH, as the median pH for the absence lakes is significantly different from the median pH of the presence lakes at the 0.05 level (Fig. 1). In addition, 75% of the absence lakes have mean pH below 6, while almost 75% of the presence lakes have mean pH above 6. Nevertheless, the lake with the highest pH is in the absence category. This underscores comments made above that the models are not correctly specified; clearly there are factors other than pH (and the remaining water chemistry variables) that determine presence/absence.

To determine whether pH and other water chemistry variables are jointly good discriminators of brook trout presence or absence, lakes with and without brook trout populations were plotted on bivariate graphs of pH and each of the other water chemistry variables. For example, for pH and ln(calcium), clusters of lakes with and without brook trout are discernible, although separation is not complete, while presence and absence of brook trout is more clearly associated with pH changes than with calcium changes (Fig. 2). The obvious bivariate pattern in Fig. 2 is a characteristic of multicollinearity and, coupled with the separation of clusters on both the pH and calcium scales, is indicative of the difficulty of drawing causal conclusions in this study.

Forward stepwise maximum likelihood logistic regression was used on the four water chemistry variables, plus interaction terms, to aid in the selection and interpretation of models.

TABLE 1. Descriptive statistics for brook trout data. Selected variables grouped by presence/absence and ln-transformed. Calcium and alkalinity in μeq/L, aluminum in μg/L, and surface area in km^2.

Variable	N	Mean	SD	Minimum	Maximum
Brook trout absent					
pH	37	5.534	1.014	4.505	7.990
ln (calcium)	30	4.723	0.613	3.761	6.646
ln (aluminum)	14	5.336	0.543	3.912	6.040
ln (alkalinity)[a]	47	5.169	0.464	4.620	7.098
ln (surface area)	48	−1.518	1.804	−4.828	3.340
Brook trout present					
pH	40	6.398	0.726	4.820	7.503
ln (calcium)	35	5.039	0.465	4.174	5.956
ln (aluminum)	7	4.408	1.504	2.303	5.991
ln (alkalinity)[a]	52	5.402	0.361	4.812	6.196
ln (surface area)	54	−1.592	1.685	−4.423	3.061

[a] Alkalinity shifted by +150 to create strictly positive values.

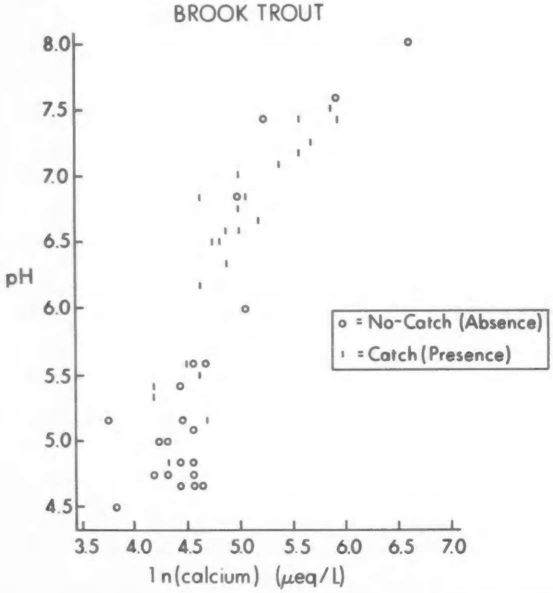

FIG. 2. pH−calcium bivariate plot for lakes with brook trout "absent" (0) and "present" (1).

TABLE 2. Statistics for the brook trout logistic function models.

Model	Variable	$\hat{\beta}$	SE
$\Pr(P) = f(\mathrm{pH})$	Intercept	−6.199	1.718
($N = 77$)	pH	1.054	0.287
$\Pr(P) = f(\mathrm{pH}, \ln(\mathrm{Ca}))$	Intercept	−2.303	2.783
($N = 46$)	pH	2.493	0.842
	ln(Ca)	−2.615	1.304
$\Pr(P) = f(\mathrm{pH}, \ln(\mathrm{Ca}),$	Intercept	−49.846	26.429
$\mathrm{pH} \cdot \ln(\mathrm{Ca}))$	pH	9.191	3.909
($N = 46$)	ln(Ca)	8.010	5.905
	pH · ln(Ca)	−1.489	0.825
$\Pr(P) = f($first	Intercept	−6.839	2.385
princ. comp.)	pH	0.810	0.282
($N = 46$)	ln(Ca)	0.429	0.149

Classification for $\Pr(P) = f(\mathrm{pH}, \ln(\mathrm{Ca}))$

	Predicted		
Observed	Absence	Presence	Total
Absence	18	5	23
Presence	4	19	23
Total	22	24	46

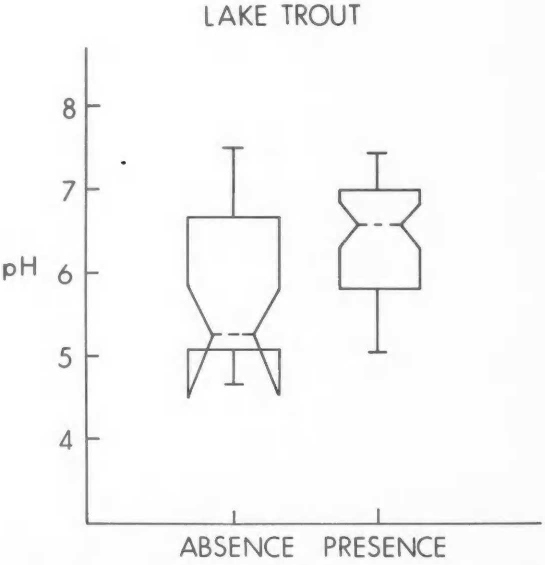

FIG. 3. pH box and whisker plots for lakes with lake trout "absent" and "present."

Using a significance level of 0.05 as the inclusion/exclusion criterion, we fit three models, one at each step (Table 2). The best of the models, based on the classification error criterion (discussed below), is the two-variable model:

$$\text{(3)} \quad \begin{array}{l} \hat{\Pr}(P) = 1/[1 + \exp\{2.303 - 2.493\,\mathrm{pH} + 2.615\,\ln(\mathrm{Ca})\}] \\ \qquad\qquad\qquad\qquad (2.783) \quad (0.842) \qquad\quad (1.304) \end{array}$$

where $\hat{\Pr}(P)$ is the response variable "probability of presence" and the numbers in parentheses below the model parameters are the standard errors. This model was fitted to a 46-lake subset of FIN which contained 23 observed absences and 23 observed presences. A somewhat optimistic measure of the effectiveness of Equation 3 for classification results when the lakes used to parameterize the two-variable logistic model are classified by the model using $\hat{\Pr}(P) = 0.5$ as the presence/absence cutoff (see Table 2, bottom). The percentage of these lakes that are correctly classified is expected to be optimistically high because the same data set is being used to both estimate parameters and assess prediction error (Efron 1986). Alternative measures of classification error are discussed in the next section.

The sample correlation coefficients between pairs of predictor variables for the three models range from 0.87 to 0.97,

TABLE 3. Descriptive statistics for lake trout data. Selected variables grouped by presence/absence and ln-transformed. Calcium and alkalinity in μeq/L, aluminum in μg/L, and surface area in km^2.

Variable	N	Mean	SD	Minimum	Maximum
Lake trout absent					
pH	17	5.798	0.939	4.725	7.503
ln (calcium)	11	4.759	0.462	4.172	5.892
ln (aluminum)	6	4.940	0.216	4.700	5.347
ln (alkalinity)[a]	17	5.172	0.375	4.852	6.194
ln (surface area)	17	−0.452	1.229	−2.561	1.763
Lake trout present					
pH	15	6.443	0.691	5.107	7.400
ln (calcium)	9	5.051	0.454	4.443	5.956
ln (aluminum)	4	4.704	1.180	3.689	6.273
ln (alkalinity)[a]	15	5.346	0.326	4.965	6.137
ln (surface area)	15	−0.530	1.208	−2.254	1.744

[a] Alkalinity shifted by +150 to create strictly positive values.

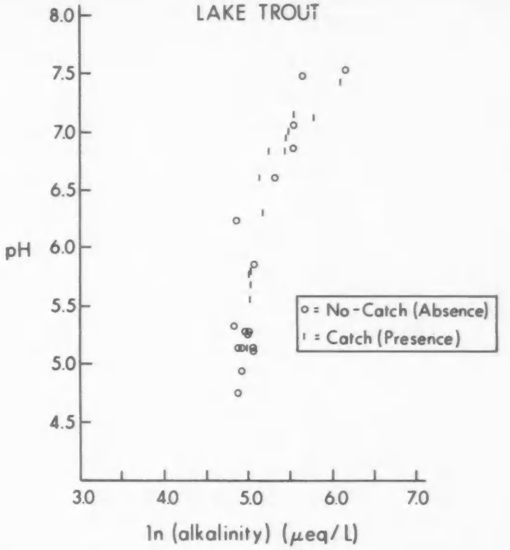

FIG. 4. pH−alkalinity bivariate plot for lakes with lake trout "absent" (0) and "present" (1). Alkalinity shifted by +150 to create strictly positive values.

indicating multicollinearity. As a consequence of the multicollinearity, the standard errors for the parameters grow substantially in magnitude as additional variables enter in the stepwise regression analysis (Table 2). In addition, as expected, the absolute values of the parameter estimates increase in magnitude as the number of (correlated) predictor variables increases. To be specific, the parameter for pH is 1.054 in the one-variable model, 2.493 in the two-variable model, and 9.191 in the three-variable model. For the three-variable model, this results from one extremely small eigenvalue ($\lambda_3 = 0.0024$), which, when considered in light of Equation 2, may be expected to cause parameter estimates high in absolute value.

For the models in Table 2, the parameter for each variable must be interpreted as being conditional on the other variables in the model. Thus, when multicollinearity is present, the parameters may appear nonsensical unless they are stated as conditional on the variables in the model. For example, the parameters in Equation 3 imply that a greater probability of brook trout absence is associated with low pH and high calcium concentrations. This seems nonsensical, since scientists generally believe that high calcium concentrations are beneficial to fish survival. However, the model is actually indicating that in a study of data from many lakes (not data from one lake over time), when the effect of pH is accounted for, lakes with higher concentrations of calcium are less likely to support a population of brook trout than are lakes with lower calcium concentrations. If the objective of model development is identification of the best overall predictive model for lake classification, then the potential for incorrect interpretation, while disconcerting, should not in itself cause elimination of any of the models from consideration. Final model selection is discussed in the next section.

One possible solution to the problems created by multicollinearity is to create one or more principal components from the predictor variables and use these orthogonal components as new independent variables in a logistic regression model. This was done for the two-variable model. The first principal component explained 95.3% of the variance in the two variables (pH and ln(calcium)), with positive component weights from each variable. The logistic regression model fitted with this component as the single independent variable appears reasonable (Table 2), as the signs on the parameter estimates for pH and ln(calcium) are the same and both parameters are significant at the 0.05 level. A nearly identical model was fitted using ridge regression with an appropriately chosen ridge parameter. Further discussion of this model, and recommendations on final model choice, are deferred to the next section.

In the development of a presence/absence model for a second species, lake trout, results similar to that for brook trout were found in an analysis of lake trout data in FIN. The box and whisker plot (Fig. 3) shows a significantly lower median pH for the absence group, yet the lake with the highest pH is in the absence group. Other water chemistry variables appear to be somewhat effective in the discrimination of presence and absence lakes, based on a comparison of means (Table 3). A bivariate pH versus ln(alkalinity) plot, with coded presence and absence lakes (Fig. 4), shows a strong correlation between these two variables, and clusters of presence and absence lakes are apparent. Both pH and alkalinity appear to be useful discriminators between the presence and absence lakes; however, the overlap of clusters on either the pH or ln(alkalinity) scale and the strong bivariate relationship between pH and ln(alkalinity) make precise causal conclusions from these analyses unwise.

Forward stepwise maximum likelihood logistic regression was used to fit a sequence of models from the water chemistry variables. The resulting models (Table 4) sequentially include the variables pH, ln(alkalinity), and an interaction term. Classification error was estimated, as before, based on the percentage of lakes in the data set that were misclassified by each model (see Table 4, bottom). The best model contains a pH−alkalinity interaction term which was found to substantially improve the overall model statistics (e.g. model chi-square and apparent classification error).

Sample correlation coefficients among the three variables in the models in Table 4 range from 0.88 to 0.98, indicating multicollinearity. This problem is also apparent in its effect on the increase in absolute value of the parameter estimates (small-

TABLE 4. Statistics for the lake trout logistic function models.

Model	Variable	$\hat{\beta}$	SE
$\Pr(P) = f(\text{pH})$	Intercept	−5.771	2.831
($N = 32$)	pH	0.923	0.457
$\Pr(P) = f(\text{pH}, \ln(\text{Alk}))$	Intercept	1.504	7.335
($N = 32$)	pH	1.832	1.002
	ln(Alk)	−2.444	2.319
$\Pr(P) = f(\text{pH}, \ln(\text{Alk}),$	Intercept	−233.424	127.584
$\text{pH} \cdot \ln(\text{Alk}))$	pH	32.388	16.648
($N = 32$)	ln(Alk)	45.662	26.091
	pH · ln(Alk)	−6.320	3.420
$\Pr(P) = f$(first	Intercept	−4.350	2.233
princ. comp.)	pH	0.235	0.128
($N = 32$)	ln(Alk)	0.091	0.050
	pH · ln(Alk)	0.073	0.040

Classification for $\Pr(P) = f(\text{pH}, \ln(\text{Alk}), \text{pH} \cdot \ln(\text{Alk}))$

	Predicted		
Observed	Absence	Presence	Total
Absence	13	4	17
Presence	4	11	15
Total	17	15	32

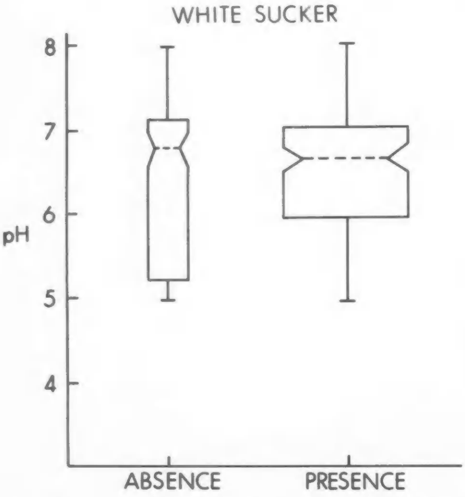

FIG. 5. pH box and whisker plots for lakes with white sucker "absent" and "present."

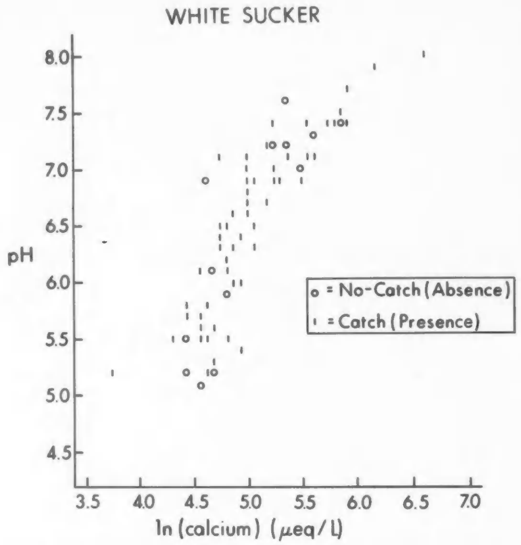

FIG. 6. pH−calcium bivariate plot for lakes with white sucker "absent" (0) and "present" (1).

est eigenvalue = 0.00026) and the increase in standard errors as the number of predictor variables increases. Additional related consequences of multicollinearity are the signs on the parameter estimates (e.g. the minus sign on ln(Alk) in the two-variable model) that appear inconsistent with theory and combinations of predictor variables that yield counterintuitive predictions (e.g. lakes with both high pH (>7.3) and high alkalinity (>300 μeq/L) are assigned lower predicted probability of presence by the three-variable model than are lakes with slightly lower values for these variables).

Principal components regression and ridge regression were applied to the lake trout data to provide insight on the multicollinearity problem and possibly to provide one or more candidate models. The first principal component from the three predictor variables explained 98.8% of the variance, so this component was used as a single predictor variable in the estimation of a new logistic regression model. The result (see Table 4) is a model with positive signs for all parameters (except the intercept); a similar model was also fitted using ridge regression with an appropriately chosen ridge parameter. In the next section, we consider this model and the others in Table 4 in the selection of a good predictive model for the classification of lakes according to the presence of lake trout.

For the other two fish species, the relationships between fish presence/absence and lake water chemistry are not well defined within the FIN data set. While pH is effective for the discrimination of brook and lake trout presence/absence lakes, it is not effective in discriminating white sucker presence and absence lakes (Fig. 5). Calcium and alkalinity are also not effective discrimination variables, based on the lack of difference between means for the presence and absence lakes (Table 5). In fact, a graph of pH versus ln(calcium) with coded presence/absence lakes (Fig. 6) shows no meaningful clusters of presence or absence lakes.

One variable that does appear to separate white sucker presence/absence lakes is aluminum, as the difference between the ln(aluminum) means for the presence and absence groups is larger for white sucker than for either brook trout or lake trout (compare Tables 1, 3, and 5). When this is presented graphically on a bivariate plot with pH (Fig. 7), two clearly defined clusters appear, one containing only absence lakes and the other containing only presence lakes. A logistic regression model fitted to this sample would classify all 24 lakes in this sample correctly. However, the sample is small, particularly for the absence class (only five lakes), and the sample tends to represent high-elevation, acidified lakes. In our view, this sample is inadequate for the purpose of model development, so no

TABLE 5. Descriptive statistics for white sucker data. Selected variables grouped by presence/absence and ln-transformed. Calcium and alkalinity in μeq/L, aluminum in μg/L, and surface area in km^2.

Variable	*N*	Mean	SD	Minimum	Maximum
White sucker absent					
pH	35	6.344	0.948	4.930	7.960
ln (calcium)	23	5.192	0.488	4.407	5.922
ln (aluminum)	5	5.029	0.221	4.868	5.394
ln (alkalinity)[a]	46	5.504	0.430	4.934	6.601
ln (surface area)	50	−0.912	1.777	−3.730	4.681
White sucker present					
pH	110	6.527	0.731	4.910	7.990
ln (calcium)	104	5.123	0.489	3.761	6.661
ln (aluminum)	19	3.979	0.772	2.303	4.963
ln (alkalinity)[a]	164	5.495	0.456	4.585	7.098
ln (surface area)	172	−1.019	1.663	−4.828	3.340

[a] Alkalinity shifted by +150 to create strictly positive values.

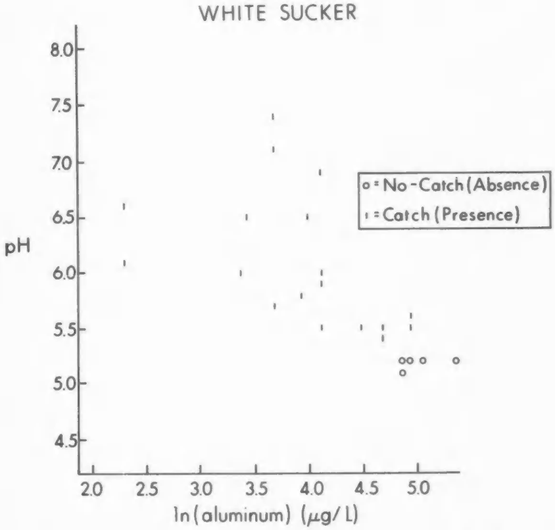

FIG. 7. pH−aluminum bivariate plot for lakes with white sucker "absent" (0) and "present" (1).

TABLE 6. Descriptive statistics for yellow perch data. Selected variables grouped by presence/absence and ln-transformed. Calcium and alkalinity in μeq/L, and surface area in km^2.

Variable	*N*	Mean	SD	Minimum	Maximum
Yellow perch absent					
pH	15	6.701	0.905	4.722	7.689
ln (calcium)	6	5.494	0.278	5.010	5.881
ln (alkalinity)[a]	17	4.959	0.727	3.301	6.253
ln (surface area)	19	−0.513	2.260	−4.830	3.055
Yellow perch present					
pH	33	6.616	0.779	4.908	7.962
ln (calcium)	37	5.279	0.461	4.541	6.180
ln (alkalinity)[a]	61	4.861	1.089	0.000	6.593
ln (surface area)	64	0.138	1.611	−3.911	3.343

[a] Alkalinity shifted by +150 to create strictly positive values.

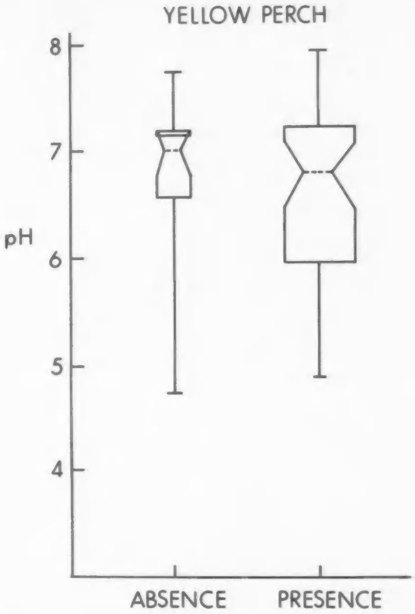

FIG. 8. pH box and whisker plots for lakes with yellow perch "absent" and "present."

model was fitted. When additional data become available, this relationship should be reevaluated.

A similar lack of association is evident upon examination of the yellow perch data. There is little separation between the box and whisker plots for pH (Fig. 8), and of perhaps greater significance, the median pH for the absence group lakes is actually higher than that for the presence group lakes. A lack of separation between the means for ln(calcium) and ln(alkalinity) indicate that these variables are not effective discriminators of presence and absence (Table 6); no aluminum data were available. Bivariate plots with coded presence/absence lakes (see Fig. 9) show no meaningful clustering of presence and absence lakes. Given this evidence, no model is proposed for yellow perch.

Classification Errors and Model Selection

When a single data set is used to specify a model, estimate the model parameters, and provide estimates of prediction error, the result is that estimates of parameter error and prediction error tend to be smaller than the true (but unknown) values (Judge et al. 1985; Efron 1986). To address this problem, classical statisticians have suggested that a data set be split for separation of these tasks. In the specific case of prediction error estimation, statisticians have proposed approaches such as cross-validation, the jackknife, and the bootstrap (see Efron 1986). All of these procedures are intended to address the problem of bias in conventional error statistics when, in effect, too much is asked of a single data set.

For classification models resulting from the application of logistic regression or discriminant analysis, statisticians (e.g. Snapinn and Knoke 1984) have considered the aforementioned approaches as well as selected probability models for the estimation of the true classification error rate. In this study, we

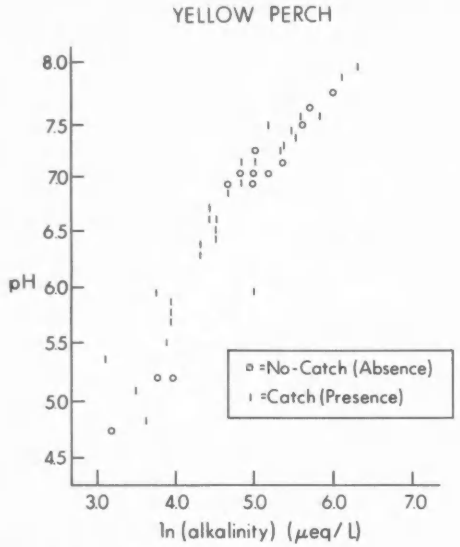

FIG. 9. pH−alkalinity bivariate plot for lakes with yellow perch "absent" (0) and "present" (1). Alkalinity shifted by +150 to create strictly positive values.

applied a normal probability model recommended by Snapinn and Knoke to estimate the true classification error for a simple discriminant function model. However, we focused our efforts on the application of a method recently proposed and evaluated by Efron (1986) for the estimation of classification error bias in logistic regression.

Efron (1986) proposed an analytical expression for expected bias in the apparent error rate in logistic regression. The apparent error rate is the proportion of cases (lakes) used to parameterize the logistic model that are incorrectly classified by that model (e.g. the proportion of incorrectly classified lakes in the classification tables in Tables 2 and 4); this is the error rate typically cited as a measure of classification error in studies involving logistic regression and discriminant analysis. Efron's expression is a maximum likelihood estimator for the expected optimism, or bias, in the apparent classification error rate. Sampling experiments presented by Efron exhibit good performance for this expression in comparison with cross-validation and the bootstrap (which is expected to yield similar results, but at a cost of considerably greater computational effort). Efron's expression for the expected bias ($\omega(\hat{\Pr}(P))$ in the apparent error rate is

$$(4) \quad \omega(\hat{\Pr}(P)) = (2/n) \sum_{i=1}^{n} \{[\hat{\Pr}(P)_i][1 - \hat{\Pr}(P)_i]\hat{\phi}_i \sqrt{\hat{d}_i}\}$$

where

$$(5) \quad \hat{\phi}_i = (2\pi)^{-\frac{1}{2}} \exp[-\tfrac{1}{2}(\hat{\beta}_k x_{ik})^2 (1/\hat{d}_i)]$$

$$(6) \quad \hat{d}_i = \hat{\mathrm{Var}}(\hat{\beta}_k x_{ik})$$

for cases (lakes) $i = 1, \ldots, n$, a P/A classification cutoff of $\hat{\Pr}(P) = 0.5$, where a circumflex represents an estimated value and where $\hat{\beta}_k x_{ik}$ has been defined as the exponential argument in the logistic model (Equation 1).

Application of Equations 4−6 to the four brook trout models (from Table 2) yields an estimate of expected bias, which, when added to the apparent error, provides an estimate of the expected true classification error (Table 7). The pH−ln(calcium) two-variable model has the lowest expected true error. It is interesting that the bias increases with the number of predictor variables, but this increase is not as great for the principal component model (which effectively has a single variable during estimation). On the basis of the expected true error alone, the pH−Ca model is to be preferred, followed by the principal component model.

TABLE 7. Classification error for the brook trout logistic function models.

Model	N	Apparent error (%)	Expected bias (%)	Expected true error (%)
$\Pr(P) = f(\mathrm{pH})$	77	27.3	0.9	28.2
$\Pr(P) = f(\mathrm{pH}, \ln(\mathrm{Ca}))$	46	19.6	2.7	22.3
$\Pr(P) = f(\mathrm{pH}, \ln(\mathrm{Ca}), \mathrm{pH} \cdot \ln(\mathrm{Ca}))$	46	23.9	3.6	27.5
$\Pr(P) = f$(first princ. comp.)	46	23.9	1.6	25.5

Model selection, however, is not quite that simple. The pH model in Table 7 was fitted and evaluated using a larger data set than was available for the other three models, as the requirement that calcium data also be used reduced the size of the sample. When this pH model is evaluated using the pH−Ca data set, the apparent error is 23.9%, the expected bias is 1.3%, and the expected true error is 25.2%; these are quite similar to the error terms for the principal component model.

Which model should be selected for predictive classification purposes? The three-variable (interaction) model can be eliminated both on the basis of high expected true error and counterintuitive predicted probabilities (as noted in the last section). The two-variable pH−Ca model has the lowest expected true error, yet with a minus sign on the calcium parameter, the model associates increasing calcium concentrations with decreasing probabilities of brook trout presence. This is counterintuitive, but the data set itself contains lakes with apparently counterintuitive observations (e.g. not all low pH lakes have no brook trout). As a result, the model is parameterized to fit these apparent idiosyncrasies in the data set, yielding the lowest apparent error (based on correct classification of only two additional observations beyond those correctly classified using the principal component model).

Given a structure and predictions that on occasion are at variance with current scientific knowledge, the pH−Ca model is rejected at this point in favor of the other two models. The choice between the pH model and the principal component model may be based largely on the desire for a one-variable or two-variable model. The advantage to the one-variable (pH) model is that to apply the model, estimates are needed only for pH; the advantage to the principal component model is that it also accounts for calcium, which is known to be important in fish survival. In the long run, model selection should be improved with a Bayesian approach substituting for the ad hoc combination of scientific knowledge and data-based models presented above.

For the four candidate lake trout models, the apparent error and expected bias (Table 8) are both larger than for the brook trout models, resulting in larger estimates of the true error. All models are based on the same 32-lake data set. For lake trout,

TABLE 8. Classification error for the lake trout logistic function models.

Model	N	Apparent error (%)	Expected bias (%)	Expected true error (%)
$\Pr(P) = f(\text{pH})$	32	31.3	4.0	35.3
$\Pr(P) = f(\text{pH}, \ln(\text{Alk}))$	32	34.4	5.5	39.9
$\Pr(P) = f(\text{pH}, \ln(\text{Alk}), \text{pH} \cdot \ln(\text{Alk}))$	32	25.0	6.2	31.2
$\Pr(P) = f$(first princ. comp.)	32	31.3	4.5	35.8

the pH−alkalinity interaction term was found to substantially reduce the apparent percent classification error from 31.3% (for the pH model) to 25.0% (for the interaction model); this is somewhat misleading in that the 6.3% error reduction is due to correct classification of only two additional lakes in the small 32-lake data set.

As noted in the previous section, multicollinearity results in counterintuitive predictions and sign (on alkalinity) for the two-variable (pH−alkalinity) lake trout model. This problem, coupled with the high expected true error for the two-variable model, effectively eliminates this model from consideration for predictive classification. No clear choice emerges from the other three models, however. The three-variable (interaction) model does yield low (<0.50) predicted probability of presence at high pH (>7.3) and high alkalinity (>300 μeq/L); outside this region the model predictions conform to scientific beliefs. Since species presence due to acidity in this relatively high pH−alkalinity region is not in question, the modeler may choose to use this model outside this region only. The one-variable (pH) and principal component models have no apparent interpretation problems, but do have a higher expected true error than does the three-variable model. The pH model, of course, has the advantage that only a single variable needs to be predicted in order to apply this model in an assessment of lake acidification management options. Some final thoughts on future model selection approaches are offered in the next section.

As an alternative for classification error estimation, discriminant analysis was used to estimate the parameters for the logistic model so that a procedure recommended by Snapinn and Knoke (1984) could be applied. The model was developed to predict the probability of presence of brook trout as a function of pH; this model was chosen because it provided the largest single-variable data set for the most effective test of the equal covariance and normality assumptions necessary for inference with discriminant analysis. At approximately the 0.05 level, neither assumption could be clearly rejected, based on an F-test and a Kolmogorov−Smirnov test, respectively. Thus, discriminant analysis was used to fit a logistic function model, with the following result:

$$(7)\quad \hat{\Pr}(P) = \frac{1}{1 + \exp(7.06 - 1.18\ \text{pH})}.$$

This model has an apparent classification error (based on the error of classification for the data set used to parameterize the model) of 27.3%.

Given the normal discriminant model, we can estimate the true classification error using an approach (based on the estimated separation between groups and the cumulative normal distribution function; Lachenbruch 1975) that was shown by Snapinn and Knoke (1984) to be preferred under conditions similar to those here. This results in a classification error rate of 30.6%, which is probably more representative (than is 27.3%; see Table 7) of the true error rate for the discriminant model.

Given concern for errors-in-variables, it was of interest to use the normal discriminant model and estimate the improvement in presence/absence discrimination if the pH observation error is eliminated. This error was estimated to be about ±0.34 pH unit in Reckhow et al. (1985). When the pH observation error is removed by subtracting variances and estimating the change in the separation between the presence and absence groups, the expected classification error (based on the normal approximation) drops from 30.6 to 29.1%. This reduction of only 1.5% suggests that shortcomings in the presence/absence model may be due more to model misspecification than to errors in basic data collection.

Conclusions and Future Work

In this study of empirical model specification and parameter estimation, it has been demonstrated that an historical data set (FIN) does contain useful information on fish species response to lake acidification. Models relating species presence/absence, for brook trout and lake trout, have been fitted to acidification-related lake water chemistry variables. Relationships for two other species, white sucker and yellow perch, were not observed in the data, so no models were parameterized.

Improvements in these models will likely result from a combination of more and better data, new scientific insights, developments in process-oriented models that can be incorporated into regional relationships, and effective application of useful statistical methods. In this latter category, a Bayesian approach has been proposed (Reckhow et al. 1985) to pool experimental data with observational data sets (like FIN) to improve parameter estimation. A Bayesian approach is also likely to be useful as an alternative to the ad hoc combination of prior beliefs and data-based models that was employed in the previous section in the selection of models. Ultimately, however, informative data and scientific knowledge will lead to the improvements in these models; effective statistical methods merely help the modeler extract the information and express it in a useful form.

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Effect of Dietary Cadmium on Growth, Survival, and Tissue Concentrations of Cadmium, Zinc, Copper, and Silver in Juvenile American Lobster (*Homarus americanus*)

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Chou, C. L., J. F. Uthe, J. D. Castell, and J. C. Kean. 1987. Effect of dietary cadmium on growth, survival, and tissue concentrations of cadmium, zinc, copper, and silver in juvenile American lobster (*Homarus americanus*). Can. J. Fish. Aquat. Sci. 44: **1443–1450.**

The addition of as much as 45 mg Cd/kg to a crab-based diet did not significantly affect growth or survival of juvenile American lobster (*Homarus americanus*) fed for 17 wk. The addition of Cd to the crab-based diet destroyed the highly significant relationship observed in nature between Cu and Ag concentrations in the digestive gland. Cd, Zn, and Cu in the crab-based diet were taken up by the digestive gland more efficiently than these metals added to the casein-based diet. Cu, Zn, and Ag concentrations in tail muscle were not affected by dietary Cd. Tissue mean molar ratios of metals (normalized to Cu and Ag) and dietary Cd ic crab-based diets showed a significant ($p < 0.05$) logarithmic relationship between dietary Cd levels and Cu/Ag (or Ag/Cu) ratios in both the tail muscle and digestive gland and Cd/Cu and Cd/Ag ratios in the digestive gland. A linear relationship was observed between Cd levels in crab-based diets and Cd/Cu, Cd/Ag, and Zn/Ag ratios in tail muscle. The absence of ascorbic acid (vitamin C) in the diet increased the uptake of Cd by the digestive gland and had an interactive effect on mean molar ratios of Cd, Cu, and Ag in the digestive gland.

L'ajout d'au plus 45 mg/kg de Cd à un régime à base de crabe n'a pas eu une incidence nette sur la croissance ou la survie du homard américain (*Homarus americanus*) juvénile nourri pendant 17 sem. L'ajout de Cd au régime à base de crabe a détruit la relation très significative observée en milieu naturel entre les concentrations de Cu et d'Ag dans la glande digestive. Le Cd, le Zn et le Cu présents dans les aliments à base de crabe ont été plus efficacement absorbés par la glande digestive que ceux ajoutés aux aliments à base de caséine. Les concentrations de Cu, de Zn et d'Ag dans le muscle de la queue n'ont pas été touchées par le Cd ajouté. Les rapports molaires moyens entre les métaux présents dans les tissus (étalonnés par rapport au Cu et à l'Ag) et le Cd ajouté aux aliments à base de crabe ont montré une relation logarithmique significative ($p < 0,05$) entre, d'une part, les teneurs en Cd alimentaire et les rapports Cu/Ag (ou Ag/Cu) dans le muscle de la queue et la glande digestive et, d'autre part, les rapports Cd/Cu et Cd/Ag dans la glande digestive. On a observé une relation linéaire entre les teneurs en Cd dans les aliments à base de crabe et les rapports Cd/Cu, Cd/Ag et Zn/Ag dans le muscle de la queue. L'absence d'acide ascorbique (vitamine C) dans le régime a stimulé la captation du Cd par la glande digestive et a eu une incidence interactive sur les rapports molaires moyens du Cd, du Cu et de l'Ag dans la glande digestive.

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Shellfish accumulate high levels of heavy metals, especially cadmium (Cd), in their tissues. Specifically, levels in excess of 500 mg Cd/kg (wet weight) have been reported in digestive gland from American lobster (*Homarus americanus*) (Uthe et al. 1982) captured in Belledune Harbour, New Brunswick, Canada. Such high Cd concentrations with no demonstrable deleterious effects on the lobster (Haya et al. 1980, Odense and Annand 1980; Uthe et al. 1982) makes it tempting to postulate a biological role for Cd in lobster. The effects of high Cd levels in lobster diet on growth and other parameters have not been established.

Highly significant relationships between silver (Ag) and copper (Cu) levels in digestive gland and claw muscle of wild American lobsters were demonstrated (Chou and Uthe 1978). Studies by Chou et al. (1981) on juvenile American lobsters showed a detoxification effect of Ag on Cu toxicity which was associated with an improvement in growth and survival. This study was undertaken to determine whether tissue levels of zinc (Zn), Cu, and Ag in juvenile lobsters would be affected by increasing levels of dietary Cd presented in both a crab protein- and casein-based diet. Survival and growth were investigated as well.

Materials and Methods

Triplicate trays, each containing 20 juvenile lobsters (average initial weight 99.64 ± 28.12 mg(SD) in individual compartments, were assigned to one of eight dietary treatments with varying Cd levels (0, 2.5, 7.5, 10, 15, 25, and 45 mg Cd/kg diet). In this experiment, two diets were used: one based on

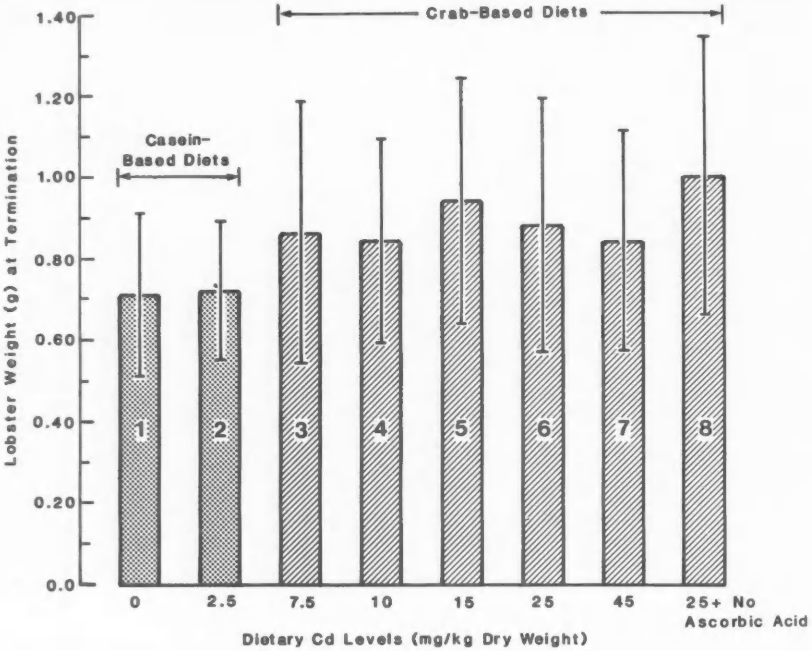

FIG. 1. Final mean weights (±SD) of juvenile lobsters fed either crab- or casein-based diets with various levels of added Cd.

TABLE 1. Dietary compositions.

Constituent	% by weight
Casein (or crab protein)	50
Gelatin	10
Corn starch	5
α-Cellulose	8.8
Mineral mix	5
Soy–lecithin	6
Cod liver oil	10
D-Glucosamine	1
Cholesterol	1
Vitamins (0.12% ascorbic acid)	2.2

Cd diet	Total Cd	Added Cd[a]	Cu (mg/kg)[b]	Zn[b]	Ag[b]
Casein-0.0	0.0	0.0	16.7[c]	0.0	0.0
Casein-2.5	2.5	2.5	16.7[c]	60[d]	0.0
Crab[e]-7.5	7.5	2.5	76.7	155	4.10
Crab[e]-10	10.0	5.0	76.7	155	4.10
Crab[e]-15	15.0	10.0	76.7	155	4.10
Crab[e]-25	25.0	20.0	76.7	155	4.10
Crab[e]-45	45.0	40.0	76.7	155	4.10
Crab[e]-25[f]	25.0	20.0	76.7	155	4.10

[a] Added as Cd citrate.

[b] Concentration determined by chemical analysis.

[c] Added as Cu citrate.

[d] Added as Zn citrate.

[e] The ingredients used in crab-based diet contributed 5.0 mg Cd/kg, 76.7 mg Cu/kg, 155 mg Zn/kg, and 4 mg Ag/kg.

[f] No ascorbic acid.

crab protein and the other on casein (Table 1). The crab-based diet was prepared from cooked, mechanically deboned whole rock crab (*Cancer irroratus*), subsequently extracted with isopropanol–water (70:30 v/v) and freeze-dried (Boghen et al. 1982). Vitamin and mineral mixes were added (Kean et al. 1985). The ingredients used in the crab-based diet contributed 5.0 mg Cd/kg, 76.7 mg Cu/kg, 155 mg Zn/kg, and 4.1 mg Ag/kg. Both casein diets, with 0.0 and 2.5 mg of added Cd, respectively, were prepared as experimental controls for comparison with Cd-fortified crab-based diets. The addition of 16.7 mg Cu/kg to casein diets was required for normal lobster growth (Chou et al. 1981). The 2.5 mg Cd/kg casein-based diet was also supplemented with 60 mg Zn/kg to match the Zn content of the crab protein diet. To investigate the possible interaction between dietary ascorbic acid and trace metals, a crab-based diet containing 25 mg Cd/kg was prepared without ascorbic acid. Concentrations of Cd, Cu, Zn, and Ag in the diets are shown in Table 1.

After feeding ad libitum three times per day (once per day on weekends) for 17 wk, the lobsters were starved for 48 h and the digestive gland and tail muscle were removed, weighed, pooled (three to five animals per sample), and digested in 5 mL of concentrated HNO_3 for Cd, Zn, Cu, and Ag determinations. Digestive gland Cd, Cu, Zn, and tail muscle Cu and Zn were determined by flame atomic absorption spectrophotometry (AAS) equipped with deuterium arc background correction. Digestive gland Ag and tail muscle Cd and Ag were determined by graphite furnace AAS equipped with a HGA 2100 controller and deuterium arc background correction.

To minimize the effect of the feeding behaviour and to compare trace metal distributions with varying protein sources

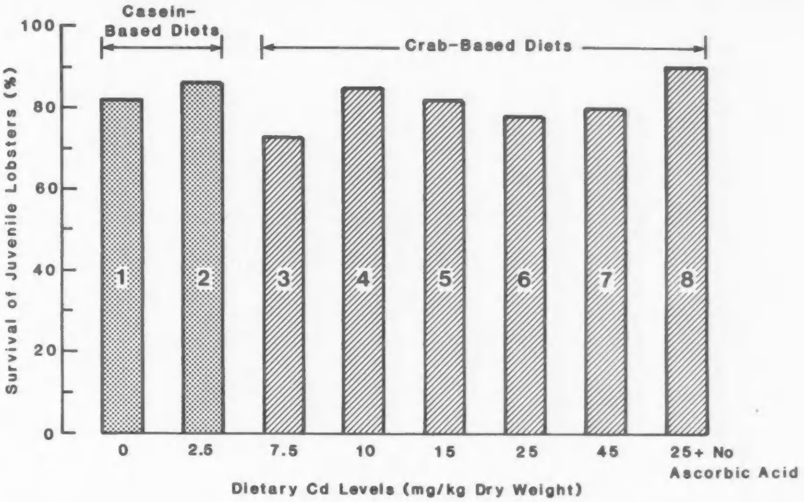

FIG. 2. Survival of juvenile lobsters fed for 17 wk on either a crab- or casein-based diet with varying levels of Cd.

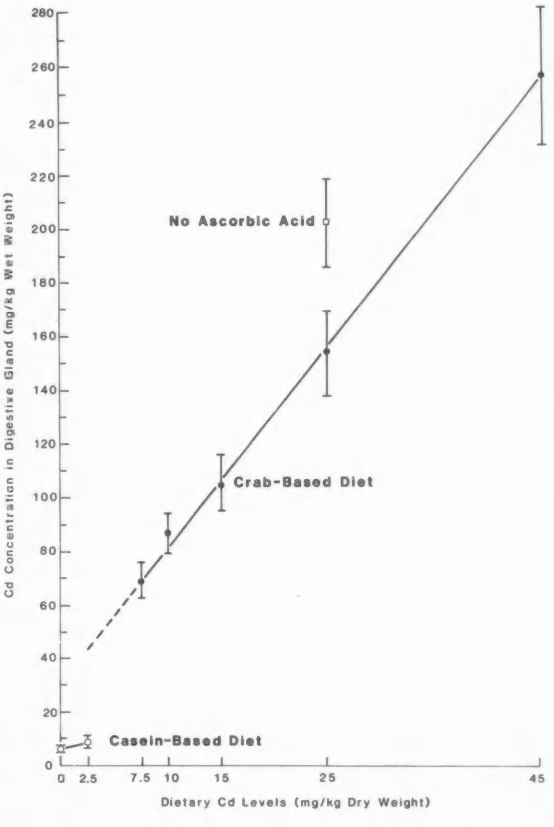

FIG. 3. Mean concentration of Cd (±SD) in digestive gland of lobsters fed for 17 wk on either a crab- or casein-based diet with varying levels of Cd.

and dietary metal levels, molar ratios of tissue metals were normalized to Cu and Ag.

Statistical analyses were conducted using the Standard and Statistical Pacs on a Hewlett-Packard model 97 calculator. All data were evaluated statistically by analysis of variance at the 5% level.

Results

Growth and Survival

Varying dietary Cd concentrations in the crab-based diets resulted in no significant differences in the growth (Fig. 1) or survival (Fig. 2) of lobsters. The presence or absence of ascorbic acid in diets with 25 mg Cd/kg had no significant effect on growth (Fig. 1) or survival (Fig. 2) of lobsters. Lobsters fed a crab-based diet generally grew better than lobsters fed a casein-based diet, but the difference was not significant (Fig. 1).

Tissue Cadmium Concentrations

The concentration of Cd in digestive gland from lobsters fed crab-based diets increased linearly with the increasing levels of dietary Cd (Fig. 3) and was described by the equation

$$\text{Mean } [\text{Cd}]_{\text{digestive gland}} = 57.13 + 12.46\ [\text{Cd}]_{\text{diet}}.$$

The coefficient of determination (r^2) was 0.999. This implies that the maximum rate of Cd uptake by this gland had not been reached despite a glandular concentration in excess of 250 mg Cd/kg wet weight being achieved with the diet containing 45 mg Cd/kg. More Cd was taken up by the digestive gland of lobsters fed a crab-based diet (naturally containing 5 mg Cd/kg) than those fed a casein-based diet.

The concentration of Cd in the tail muscle from lobsters fed crab-based diets (Fig. 4) was much less than in the digestive gland, but it was also related to dietary Cd as described by the equation

$$\text{Mean } [\text{Cd}]_{\text{tail muscle}} = 0.059 + 0.074 \log\ [\text{Cd}]_{\text{diet}}.$$

TABLE 2. Effect of protein source (crab or casein) and varying dietary Cd levels on mean concentrations (mg/kg wet wt) (±SD) of Ag, Cd, Cu, and Zn in lobster digestive gland and tail muscle.

Cd diet	[Cd]	[Zn]	[Cu]	[Ag]
Digestive gland				
Crab-45	257.9 (±26.3)	203.9 (±36.0)	660.0 (±56.1)	8.15 (±2.90)
Crab-25	154.0 (±15.7)	186.0 (±32.9)	679.6 (±103.8)	9.16 (±4.49)
Crab-25[a]	202.1 (±16.5)	221.7 (±41.4)	650.9 (±45.0)	7.60 (±4.43)
Crab-15	105.6 (±10.6)	165.1 (±23.7)	602.6 (±94.1)	10.22 (±3.61)
Crab-10	86.64 (±7.26)	195.5 (±62.6)	649.8 (±47.8)	15.46 (±5.44)
Crab-7.5	69.00 (±6.51)	190.3 (±45.9)	583.0 (±49.6)	13.28 (±4.98)
Casein-2.5	8.23 (±1.93)	36.89 (±7.97)	11.57 (±7.01)	0.067 (±0.027)
Casein-0.0	5.88 (±0.86)	13.04 (±2.67)	12.19 (±2.89)	0.089 (±0.027)
Tail muscle				
Crab-45	0.204 (±0.085)	14.11 (±1.47)	9.88 (±4.04)	0.204 (±0.102)
Crab-25	0.185 (±0.054)	13.15 (±0.74)	8.23 (±1.41)	0.160 (±0.032)
Crab-25[a]	0.205 (±0.072)	12.25 (±1.74)	7.73 (±1.65)	0.151 (±0.026)
Crab-15	0.170 (±0.041)	12.46 (±1.22)	7.13 (±0.92)	0.134 (±0.020)
Crab-10	0.088 (±0.034)	13.50 (±2.08)	9.65 (±1.65)	0.173 (±0.036)
Crab-7.5	0.059 (±0.031)	13.43 (±1.58)	8.91 (±2.63)	0.154 (±0.050)
Casein-2.5	0.030 (±0.015)	12.55 (±0.91)	6.46 (±0.87)	0.026 (±0.006)
Casein-0.0	0.028 (±0.017)	11.48 (±0.83)	7.52 (±1.55)	0.038 (±0.008)

[a] No ascorbic acid added.

TABLE 3. Analysis of variance on the digestive gland (DG) and tail muscle (TM) metal concentrations (mg/kg wet wt) in lobsters fed with varying Cd-supplemented crab-based diets (7.5−45 mg Cd/kg) and with or without ascorbic acid-supplemented diets (25 mg Cd/kg).

	Cd_{TM}	Zn_{TM}	Cu_{TM}	Ag_{TM}	Cd_{DG}	Zn_{DG}	Cu_{DG}	Ag_{DG}
	Among the varying dietary Cd treatment							
F^{a}_{observed}	7.90	1.37	1.96	1.98	212.0	1.01	2.79	3.44
	Between with and without ascorbic acid treatment at 25 mg dietary Cd/kg							
F^{b}_{observed}	0.424	3.20	0.448	0.881	37.83	3.75	0.57	2.62

[a] F calculated value is 2.84 at $p \leq 0.05$.
[b] F calculated value is 4.54 at $p \leq 0.05$.

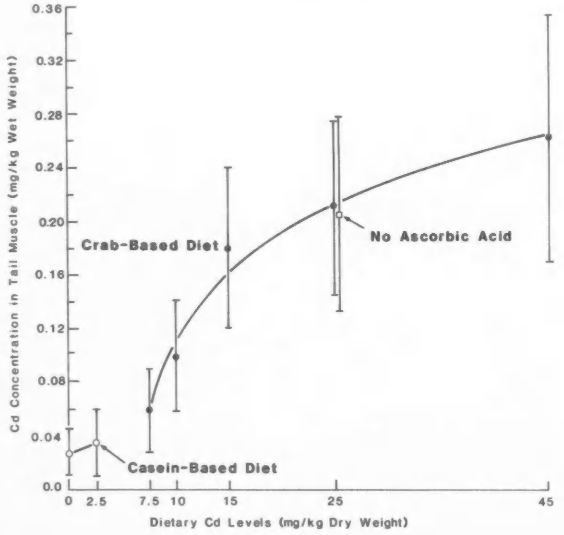

FIG. 4. Mean concentration of Cd (±SD) in tail muscle of lobsters fed for 17 wk on either a crab- or casein-based diet with varying levels of Cd.

TABLE 4. Coefficient of determination (r^2) for Ag and Cu concentrations in the lobster digestive gland (DG) or in the lobster tail muscle (TM) in relation to the varying dietary Cd treatments.

Cd diet	Cu_{DG} vs. Ag_{DG}	Cu_{TM} vs. Ag_{TM}
Crab-45	0.092	0.956
Crab-25	0.252	0.820
Crab-25[a]	0.004	0.753
Crab-15	0.058	0.801
Crab-10	0.230	0.930
Crab-7.5	0.516	0.862
Casein-2.5	0.860	0.723
Casein-0.0	0.817	0.806

[a] No ascorbic acid added.

The coefficient of determination was 0.983. However, the entrance of Cd into tail muscle may be limited, since the curve leveled off at higher dietary levels of Cd.

Lobsters fed the diet with ascorbic acid supplement had a lower concentration of Cd in the digestive gland than those fed the unsupplemented diet. The presence or absence of ascorbic acid in the diet has no effect on Cd concentration in the tail muscle.

TABLE 5. Effect of dietary protein source and varying dietary Cd level on mean molar ratios (±SD) of Cd, Ag, Cu, and Zn to Cu or Ag in lobster digestive gland and tail muscle.

Cd diet	Cd/Cu[b] ($\times 10^{-2}$)	Ag/Cu[b] ($\times 10^{-3}$)	Zn/Cu ($\times 10^{-1}$)
Digestive gland			
Crab-45	22.16 (±1.78)	7.25 (±2.33)	2.99 (±0.44)
Crab-25	13.07 (±2.42)	9.96 (±3.84)	2.75 (±0.73)
Crab-25[a]	17.61 (±1.74)[c]	6.92 (±2.20)[c]	3.33 (±0.70)
Crab-15	10.01 (±0.94)	10.07 (±3.31)	2.71 (±0.54)
Crab-10	7.57 (±0.80)	13.89 (±4.97)	2.93 (±0.96)
Crab-7.5	6.70 (±0.48)	13.21 (±4.39)	3.15 (±0.65)
Casein-2.5	47.78 (±19.45)	3.62 (±0.71)	36.41 (±13.44)
Casein-0.0	28.58 (±7.66)	3.95 (±0.69)	10.83 (±2.90)
Tail muscle			
Crab-45	1.48 (±0.43)	12.06 (±1.40)	1.52 (±0.38)
Crab-25	1.25 (±0.21)	11.40 (±1.02)	1.59 (±0.29)
Crab-25[a]	1.47 (±0.30)	11.64 (±1.25)	1.59 (±0.33)
Crab-15	0.780 (±282)	11.17 (±0.77)	1.71 (±0.12)
Crab-10	0.523 (±212)	10.09 (±0.51)	1.37 (±0.16)
Crab-7.5	0.354 (±144)	9.54 (±1.19)	1.54 (±0.32)
Casein-2.5	0.265 (±0.126)	2.31 (±0.39)	1.95 (±0.33)
Casein-0.0	0.207 (±0.110)	3.08 (±0.27)	1.56 (±0.37)

Cd diet	Cd/Ag[b]	Zn/Ag[b]	Cu/Ag[b]
Digestive gland			
Crab-45	32.17 (±6.00)	45.73 (±10.50)	147.4 (±35.58)
Crab-25	15.36 (±8.21)	32.15 (±17.94)	116.3 (±53.10)
Crab-25[a]	33.71 (±18.11)	64.40 (±39.28)	193.8 (±10.73)[c]
Crab-15	10.59 (±2.35)	28.27 (±6.17)	107.7 (±29.94)
Crab-10	7.0 (±4.85)	21.68 (±10.67)	87.78 (±51.08)
Crab-7.5	5.75 (±1.82)	25.04 (±5.32)	82.95 (±25.87)
Casein-2.5	129.2 (±47.42)	1016.0 (±399)	286.8 (±59.91)
Casein-0.0	68.01 (±22.49)	266.0 (±82.80)	236.7 (±28.67)
Tail muscle			
Crab-45	1.26 (±0.42)	127.8 (±35.13)	83.90 (±9.89)
Crab-25	1.11 (±0.24)	141.1 (±34.64)	88.34 (±7.87)
Crab-25[a]	1.28 (±0.31)	136.6 (±22.37)	78.52 (±8.55)
Crab-15	0.710 (±0.291)	154.1 (±10.32)	90.58 (±6.27)
Crab-10	0.520 (±0.002)	135.3 (±13.42)	99.36 (±5.02)
Crab-7.5	0.380 (±0.170)	164.0 (±43.32)	106.1 (±12.26)
Casein-2.5	1.00 (±0.25)	869.3 (±215.7)	444.7 (±103.3)
Casein-0.0	0.660 (±0.302)	503.7 (±95.4)	327.0 (±29.7)

[a] No ascorbic acid added to the diet.

[b] Significantly different at the 5% level from varying dietary Cd levels in crab-based diet (25 mg dietary Cd/kg, no ascorbic acid diet data not included). Tail muscle Zn/Ag data not included.

[c] Significantly different at the 5% level (n = 19) from ascorbic acid-supplemented.

Tissue Zinc Concentrations

Digestive gland Zn levels in lobsters fed the crab-based diets were approximately 4 times greater than in lobsters fed with Zn-supplemented casein diet (Table 2), although there was only 2.5 times more Zn in the crab-based diet (Table 1). Varying dietary levels of Cd had no significant effects on digestive gland or tail muscle Zn concentrations (Table 3). This implies that the tissue Zn levels are under biological control and independent of dietary Cd-induced effects. The absence of ascorbic acid in 25 mg dietary Cd/kg crab-based diet had no statistically significant effect on Zn concentrations in either tissue (Table 3), although the mean concentration of Zn was 20% lower in digestive glands of lobsters fed diets containing ascorbic acid than in lobsters not receiving this supplement.

Tissue Copper Concentrations

There were no significant differences among levels of Cu in digestive gland or tail muscle of lobsters fed crab-based diets (Table 3). Average concentrations of Cu in digestive glands of lobsters fed crab-based diets varied from 583 to 679 mg Cu/kg; levels of Cu in digestive glands of lobsters fed casein diets were approximately 12 mg Cu/kg (Table 2). Thus, Cu concentration in the digestive gland of lobsters fed crab-based diets was approximately 50 times that of lobsters fed casein-based diets

TABLE 6. Regression relationships between dietary Cd concentrations in crab-based diet (x) and molar ratio of metals to Ag or Cu (y) in lobster tail muscle and digestive gland. NS = not significant.

Y	r^2	a	b	Regression equation
Tail muscle				
Cd/Cu	0.675[a]	7.03×10^{-4}	0.831	$y = ax^b$
Ag/Cu	0.444[a]	7.54×10^{-3}	0.127	$y = ax^b$
Zn/Cu	NS	—	—	—
Cd/Ag	0.575[a]	9.23×10^{-2}	0.707	$y = ax^b$
Zn/Ag	NS	—	—	—
Cu/Ag	0.445[a]	132.56	−0.127	$y = ax^b$
Digestive gland				
Cd/Cu	0.939[a]	3.536×10^{-2}	4.080×10^{-3}	$y = a + bx$
Ag/Cu	0.272[a]	2.584×10^{-2}	−0.338	$y = ax^b$
Zn/Cu	NS	—	—	—
Cd/Ag	0.785[a]	−0.202	0.701	$y = a + bx$
Zn/Ag	0.359[a]	18.368	0.596	$y = a + bx$
Cu/Ag	0.272[a]	38.617	0.338	$y = ax^b$

[a] Significant at the 5% level.

rather than the 5 times Cu difference between the diets. This implies that Cu was more bioavailable from the crab-based diet than from the casein-based diet. The presence or absence of ascorbic acid in crab-based diets containing 25 mg Cd/kg crab-based diet had no effect on Cu levels in digestive glands or tail muscle.

Tissue Silver Concentrations

There were significant differences ($p < 0.025$) among the digestive gland Ag levels of lobster fed the crab-based diets (Table 3). The concentration of Ag in the digestive gland averaged 33% lower at 15 mg Cd/kg than at 10 mg Cd/kg diet and decreased further to an average of 44% at high dietary Cd levels up to 45 mg Cd/kg diet. Lobster fed casein-based diets containing no measurable level of Ag had Ag levels in the digestive gland of 0.067 mg Ag/kg for 2.5 mg Cd/kg diet and 0.089 mg Ag/kg for 0.00 mg Cd/kg diet. The uptake of Ag in the lobster tail muscle was not significantly affected by dietary Cd. The presence or absence of ascorbic acid in the 25 mg Cd/kg crab-based diet had no effect on Ag levels in either tissue.

Copper−Silver Relationships

In natural populations of adult lobsters there is a highly significant relationship ($r^2 \approx 1.00$) between Cu and Ag concentrations in both digestive gland and tail muscle (Chou and Uthe 1978). In the present study the coefficient of determination for the relationship in lobster tail muscle was highly significant for all diet treatments (Table 4), but in the digestive gland it was no longer significant when dietary Cd increased above 10 mg/kg. The loss of the Cu−Ag relationship in the lobster digestive gland may reflect interaction between Ag and the relatively high dietary levels of Cd and Cu.

Mean Molar Ratio of Metals to Copper or Silver

Tail muscle Zn/Cu ratios in both diets were essentially constant (Table 5). This is in agreement with the above finding that the levels of Zn and Cu in the tail muscle were unaffected by the addition of dietary Cd. Lobsters fed casein-based diets had higher digestive gland Zn/Cu ratios than those fed crab-based diets (Table 5). For example, the casein-based diet with a dietary Zn/Cu ratio of 3.59 resulted in a digestive gland Zn/Cu ratio 12 times higher than the crab-based diets with a dietary Zn/Cu ratio of 2.02, reflecting the increased bioavailability of Cu in the crab diet. Increasing dietary Cd levels in crab-based diets resulted in increasing Zn/Ag ratios in digestive gland. This is not surprising, since Ag levels in the digestive gland decreased with increasing dietary Cd levels. As expected, there was no significant relation between dietary Cd levels in crab-based diets and Zn/Ag ratio in tail muscle (Table 6).

Increasing dietary Cd levels resulted in decreasing Ag/Cu ratios in digestive gland. In contrast, tail muscle Ag/Cu ratios increased with increasing dietary Cd levels. The lack of Ag in the casein-based diet resulted in a lower mean molar ratio of Ag/Cu in both tissues than in those fed the crab-based diet. As expected, mean molar ratios of Cd/Cu and Cd/Ag in both tissues increased with increasing dietary Cd levels.

Investigations of tissue mean molar ratios of metals to Cu or Ag and dietary Cd in crab-based diets showed that there was a logarithmic relationship between dietary Cd levels and Cu/Ag (or Ag/Cu) ratios in both tissues, Cd/Cu and Cd/Ag in digestive gland, but a linear relationship with Cd/Cu, Cd/Ag, and Zn/Ag in tail muscle (Table 6).

The absence of ascorbic acid from the 25 mg Cd/kg diet (Table 5) resulted in significantly higher mean molar ratios of digestive gland Zn/Cu, Cd/Cu, Ag/Cu, Cd/Ag, and Cu/Ag than diets containing ascorbic acid, but no difference in mean molar ratios of digestive gland Zn/Cu, Zn/Ag, and molar ratios of tail muscle metals to Cu or Ag.

Discussion

The lack of a detrimental effect on growth or survival among juvenile lobsters fed a crab-based diet supplemented with as high as 45 mg Cd/kg diet was unexpected, since Cd has long been identified as a toxic element in mammals (Nordberg 1972). However, the observations that (1) high Cd concentrations are normally present in lobster digestive gland

(Freeman and Uthe 1974), (2) Cd in lobster digestive gland is not readily lost or depurated from lobsters (Uthe et al. 1980), and (3) lobsters with digestive gland Cd concentrations in excess of 300 mg/kg wet weight do not demonstrate any observable ill-effects (Haya et al. 1980; Odense and Annand 1980) suggest that Cd may have different effects in lobster than are commonly observed in mammals (Nordberg 1972).

The Cd concentration in the digestive gland was linearly related to the dietary Cd level up to at least 250 mg Cd/kg wet weight digestive gland (Fig. 3). The lack of adverse effects on growth and survival (Fig. 1 and 2) at this high level indicates that the digestive gland has a remarkable capacity for Cd uptake and storage. Unfortunately, the feeding behaviour of the animals makes it impossible to determine accurately the proportion or amount of dietary Cd taken up by the gland. Cd levels of 0.028−0.205 mg/kg wet weight in tail muscle from varying dietary Cd concentrations were comparable with those found in other marine species (Greig et al. 1977). The responses of both tail muscle Cd and digestive gland Cd to dietary Cd may indicate that Cd, unlike Cu and Zn, is not regulated by lobsters. Numerous authors have reported that Cd is not regulated by decapods (Vernberg et al. 1974; Bengtsson 1977; Wright 1977; Dethlefsen 1978; Jennings and Rainbow 1979).

Zn and Cu levels in tail muscle (Table 2) were not influenced by the high dietary Cd concentration or the protein source. This demonstrates that lobsters are able to regulate Cu and Zn. This is in agreement with Bryan's (1964) observations that (1) Zn concentrations in lobster muscle appear to be controlled, (2) lobster muscle contains very little Cu (5.7 mg/kg wet weight), and (3) no evidence was found of interaction between Zn and Cu in the digestive gland. White and Rainbow (1982) concluded from their study of decapod crustaceans exposed to dissolved Zn that *Palaemon elegans* had the ability to regulate Cu and Zn. Although dietary Cd did not affect Cu or Zn levels in tail muscle, the crab diet resulted in a higher Cu concentration in digestive gland than did the equivalent casein diet. It is obvious that components of diet, such as amino acids, and natural complexing ligands, as well as protein digestion by animals influence trace metal absorption (Kirchgessner and Grassmann 1970; McCall and Davis 1961). Ag uptake in tail muscle from the crab diet was unaffected by varying dietary Cd, and its final concentration was greater than from the casein diet. It was surprising that the digestive gland Ag levels were affected by the dietary Cd, although interaction between Ag and Cu in lobster has been observed in that Ag is involved in the detoxification of excess Cu in the diet (Chou et al. 1981).

Ag interaction with Cu metabolism has been reported for both poultry and rats, but there are relatively few studies of this trace metal in aquatic species. Hill et al. (1964) observed that Ag accentuated the Cu deficiency induced in chicks by a low-Cu basal diet. Jansen et al. (1964) found that Ag depressed growth rate, reduced packed cell volume, slightly reduced hemoglobin level, and induced cardiac enlargement in turkey poults. All of these conditions were prevented by adding 50 ppm of Cu to the diet. In contrast, we observed (Table 4) that Cd added to the crab-based diet destroyed the relationship between Cu and Ag in the digestive gland that was observed in both wild adult lobsters (Chou and Uthe 1978) and those fed the casein-based diet. The Cu−Ag relationship suggests that Cu and Ag have some type of biochemical interaction. The lack of a significant correlation between these two elements in the digestive gland of the lobster fed the higher levels of Cd suggests that the uptake of Ag and Cu by the digestive gland occurs independently, and that dietary Cd subtly affects the uptake of one or both of these metals. The maintenance of the relationship in the tail muscle suggests that some type of biochemical control on muscle levels of these elements is exerted by the animal despite the dietary Cd level.

The response of mean molar Ag/Cu ratios (Table 5), decreasing in digestive gland and increasing in tail muscle, to the addition of dietary Cd levels in the crab-based diet suggests that there is some degree of biological control of the intake of both metals in lobsters either through a detoxification mechanism or metal−metal interaction. It is not clear whether the decreasing mean molar Ag/Cu ratio in lobster digestive gland, resulting in the loss of the digestive gland Ag−Cu relationship, was due to the high intake of digestive gland Cu level or to the addition of inorganic Cd to the crab-based diet. It has been observed (Chou et al. 1981) that lobster fed 80 mg Cu/kg in a casein-based diet (without added Cd) resulted in an increased uptake of Ag by the digestive gland and maintenance of the digestive gland Ag−Cu relationship (r^2 = 0.735). This suggests that the loss of the digestive gland Ag−Cu relationship and the decreasing mean molar ratio of digestive gland Ag/Cu in this study may be attributed to metal−metal interaction including the influence of high dietary inorganic Cd supplement.

With the exception of an increase in the uptake of Cd by the digestive gland and effects on certain metal molar ratios in the digestive gland, the absence of ascorbic acid in the diet did not affect growth, survival, or the level of Zn, Cu, or Ag in the tissues or the level of Cd in the tail muscle. The increased uptake of Cd by the digestive gland in the absence of ascorbic acid may have resulted from increased leaching of Cd from the food into the surrounding water in the presence of ascorbic acid or a direct effect of ascorbic acid on the uptake of Cd. Other observations (Table 5) are that the absence of ascorbic acid in the diet of 25 mg Cd/kg caused higher mean molar ratios of digestive gland Cd/Cu, Cd/Ag, and Cu/Ag than diets containing ascorbic acid, but made no difference in mean molar ratios of all metals in tail muscle. These observations indicate that (1) the digestive gland is the main organ in lobster for the bioaccumulation of Cd, Cu, and Ag and (2) the addition of ascorbic acid to the diet plays an important role in the uptake of trace metal by the digestive gland and in the metal−metal interaction within the digestive gland. In studies of other species, it is generally observed that ascorbic acid produces a significant ameliorative effect against excessive levels of heavy metals (Van Campen and Gross 1968; Hill and Starcher 1965).

Overall, the uptake and resulting tissue levels of dietary trace metals are not simply predicted by the concentration of each element in the diet. Marked interactions occurred among the trace elements themselves, and between trace metals and other dietary constituents such as proteins used in formulating the diet. Toxic effects of dietary trace metals are difficult to predict from either dietary concentrations or, indeed, tissue concentrations of the metals. For example, we have shown that dietary Ag and Cu interact in such a manner that there is an optimum ratio of these two trace metals. It is obvious from these studies that toxicological experiments within the laboratory must consider nutritional characteristics of both the animal and the diet when interpreting results or extrapolating them to field conditions.

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Effects of Snow and Ice on the Annual Cycles of Heat and Light in Saqvaqjuac Lakes

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The annual cycles of temperature, ice and snow cover, and light are described for small Saqvaqjuac lakes (63°30′N). Summer thermal stratification occurred but was not persistent. Annual heat budgets were slightly higher than for temperate lakes and increased with increasing mean depth; latent heat of fusion constituted one half to one third of the total heat flow. Ice thickness reached a maximum of 1.6−2.2 m, depending upon winter snow cover, and a thin layer (≈5 cm) of white ice occurred in some years. Ice-out was a partial function of mean June temperature. The open-water season lasted 2−3 ms. Light attenuation through white and candled ice, snow, and water was measured. About 28% of the annual surface irradiance penetrated the unfrozen water, about half that which would have been absorbed under ice-free conditions. Extinction coefficients were inversely correlated with mean depth and positively correlated with nutrient status.

On décrit les cycles annuels de la température, de la lumière et de la couverture de glace et de neige dans certains petits lacs de Saqvaqjuac (63°30′N). Une stratification thermique estivale avait lieu mais elle n'était pas continue. Les bilans thermiques annuels étaient légèrement supérieurs à ceux observés dans des lacs tempérés et augmentaient en fonction de la profondeur moyenne; la chaleur latente de fusion représentait de une demie à deux tiers du flux thermique total. L'épaisseur de la glace a atteint un maximum de 1,6−2,2 m selon la couverture de neige et, certaines années, une mince couche de glace blanche (≈5 cm) s'est formée. Le moment de la débâcle des glaces dépendait en partie de la température moyenne en juin tandis que la saison d'eau libre durait de 2 à 3 mo. On a quantifié la réduction de l'intensité de la lumière à travers la glace en aiguilles, la glace blanche, la neige et l'eau. Environ 28 % du rayonnement superficiel annuel a pénétré les eaux non gelées, soit environ la moitié du pourcentage qui aurait été absorbé en l'absence de glace. Les coefficients d'extinction étaient en corrélation négative avec la profondeur moyenne et en corrélation positive avec la teneur en bioéléments.

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Good year-round description of light reaching phytobenthos and phytoplankton is essential for detailed modelling of primary production based upon chlorophyll and photosynthesis−irradiance curves (Fee 1973), but only Welch and Kalff (1974) have given reasonably complete data on the light climate of an arctic lake. Extensive limnological data sets have been published for only two other North American arctic sites, Barrow, Ak, and Resolute, N.W.T., site of the IBP Char Lake Program (Schindler et al. 1974). The Alaska work, reviewed in Hobbie (1973), was mostly on relatively shallow thermokarst tundra ponds and lakes which are quite different from most Canadian central and eastern arctic lakes. Other partial descriptions of ice and thermal regimes include Minns (1977) on Devon Island, Hansen (1967) for Greenland lakes, Rust and Coakley (1970) for Stanwell−Fletcher on Somerset Island, and Deane (1958) for Lake Hazen, northern Ellesmere Island; none provides comprehensive year-round observations.

During a 7-yr (1977−83) study of the effects of phosphorus and nitrogen addition to arctic lakes, many physical limnological data were collected. Here we report our observations on the annual cycles of temperatures and light in small Saqvaqjuac lakes, as affected by prolonged ice and snow cover. Saqvaqjuac station is located at 63°39′N, 90°39′W on a small inlet on the northwest coast of Hudson Bay about 40 km north of Chesterfield Inlet hamlet.

The numerous lakes at Saqvaqjuac lie in glacially scoured depressions in Precambrian granitic gneiss bedrock. The land was submerged beneath the sea to an altitude of ≈160 m above present sea level after the Wisconsinan glaciation. The present isostatic rebound rate is about 0.9 m per century (Andrews 1969). The climate is typical of the central arctic, modified by adjacent Hudson Bay, with mean July and January temperatures of 10 and −28°C, respectively. Mean annual windspeed is ≈21 $\mathrm{km \cdot h^{-1}}$ with no seasonal pattern. Mean annual precipitation is 29 cm, about half occurring as summer rainfall. A more complete description and maps of the study area may be found in Welch (1985). Companion papers pertinent to physical limnology include winter lake circulation (Welch and Bergmann 1985), mixing of meltwater beneath lake ice (Bergmann and Welch 1985), and chemical limnology (Welch and Legault 1986).

We use data from P & N Lake for most of our examples, and the physical limnology of other small, headwater lakes differs only in minor details from P & N Lake. Pertinent morphometric details for the seven Saqvaqjuac lakes discussed here are given in Table 1; all are within 4 km of camp. Background data were gathered in 1977−78. P & N Lake

TABLE 1. Morphometry of selected Saqvaqjuac lakes

Lake	Z_{mean}	Z_{max}	A_0 (ha)[a]	A_D (ha)[b]	% ice volume[c]
Jade	1.82	5.2	3.63	27.9	77
Far	3.61	8.9	3.70	16.6	46
P & N	3.28	10.2	7.09	28.6	47
Spring	2.71	7.1	6.93	30.6	56
Turkey	10.8	29.4	41.2	3725	12
Hawk	11.6	34.2	24.3	299	23
Methane North	4.3	11.3	5.3	19.3	38
Methane South	6.0	15.3	3.4		29

[a] Lake area.

[b] Drainage area excluding lake area.

[c] At 2 m ice thickness.

received 1.0 g $N \cdot m^{-2} \cdot yr^{-1}$ (as $NaNO_3$) and 0.1 g $P \cdot m^{-2} \cdot yr^{-1}$ (as H_3PO_4) during 1979–83, Far Lake received 0.1 g $P \cdot m^{-2} \cdot yr^{-1}$ during 1979–83, and Jade Lake received 2.0 g N and 0.2 g $P \cdot m^{-2} \cdot yr^{-1}$ in 1979 and 1980. Other lakes were unperturbed.

Methods

Temperature profiles were made with a thermistor coupled with an ohmmeter, calibrated against an ice bath and NBS-calibrated thermometer, to an accuracy better than 0.1° throughout the range 0–15°C. The precision of any measurement was ±0.03°, the digital readout precision of the ohmmeter. Ice thickness measurements were made routinely wherever holes were drilled, but measurements made where localized ice chip and snow buildup occurred were discarded. The maximum winter ice thickness was also surveyed in May by drilling 8–13 holes on each lake. Snow cover was routinely measured at 2-wk intervals by walking outward from the center of the lake, measuring snow depth five times at 5-m intervals, in four directions (north, east, south, and west) (total $N = 20$). Extensive annual spring snow surveys were also conducted to determine the winter maximum snow cover on each lake.

All light measurements at Saqvaqjuac were made with Licor® 185A quantum meters combined with model 192-S cosine-corrected flat plate quantum detectors which were sensitive to radiation in the 400–700 mm range (photosynthetically active radiation (PAR)). Surface (incident) solar radiation was monitored continuously at the Saqvaqjuac laboratory, recorded on strip charts, and reduced with a digitizer connected to a computer.

Light transmittance through typical wind-drifted snowpack was measured as per Welch and Kalff (1974). Because light absorbance by snow is not linear with depth (e.g. Fig. 1), mean snow cover could not be used to obtain mean light transmission. Instead we used the procedure illustrated in Fig. 2. Annual surveys showed that snow cover was thicker near shore, but since this belt of thicker snow was restricted almost entirely to areas where water depth was <2 m, the average depth of freezing, it was ignored.

Four sets of data on light transmission through white (slush) ice (Appendix) were obtained as follows. Blocks of lake ice ≈40 cm in diameter with a white ice layer at the surface were removed from the lake. The clear ("black") ice at the bottom was shaved off and the white ice layer suspended so that the top was exposed to sunlight but no light could enter the sides or bottom. A 192-S quantum sensor was held facing upward against the bottom and measurements of surface and transmitted light were made, the white ice layer thinned by shaving off the underside, and the measurements repeated.

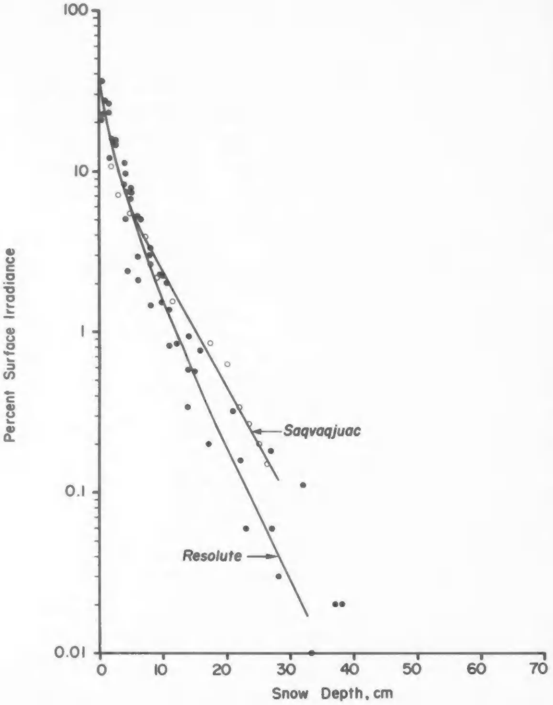
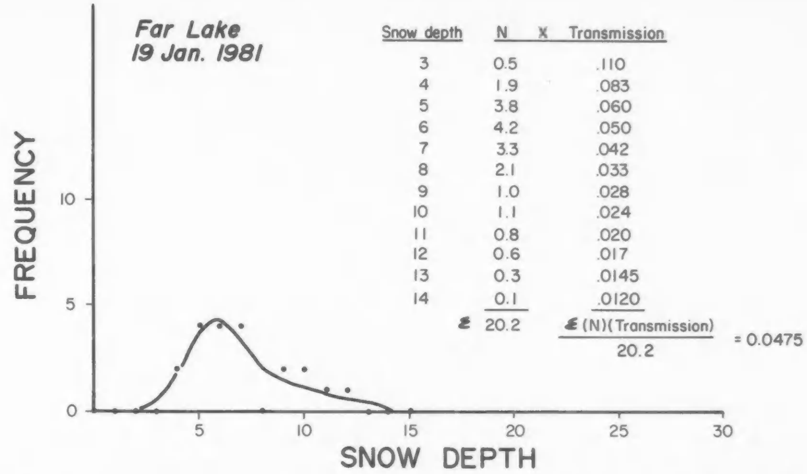


FIG. 1. Light transmission through typical wind-deposited arctic snowpack. Solid circles and lower line from Welch and Kalff (1974); data from Resolute, N.W.T. Open circles from present study. The upper line was used to calculate light penetration at Saqvaqjuac (see Methods).

Transmission of light through candled ice was measured as follows. A dive hole was cut in the center of Methane Lake South on 1 June 1980 and lines run under the ice between it and three 15-cm-diameter holes 18 m distant. A 192-S quantum sensor was inserted in a small floating raft 30 cm wide by 40 cm long by 17 cm deep, such that the sensor was held 1 cm below and normal to the underice surface. The raft with probe was then towed from the dive hole out to each small hole and back, with simultaneous surface and subice light readings being made at 1-m intervals ≥2 m from the dive hole. Ice thickness was taken separately as the mean of seven 15-cm-diameter holes drilled in a circle 60 m in diameter around the dive hole.

Light transmission through clear black ice was assumed to be the same as through pure water ($\epsilon = 0.05$) (eq. 1) (Bolsenga 1981; but see Grenfell and Perovich 1981 for spectral absorption coefficients).

Measurements of light transmission through water were made by lowering an upwardly aimed 1982-S sensor and measuring in situ radiation at 1-m intervals, corrected with simultaneous surface (deck) readings. When the lakes were ice and snow covered, an opaque cover was placed over the sampling hole (the shadow was undetectable below 3 m). Light



Snow depth	N	× Transmission
3	0.5	.110
4	1.9	.083
5	3.8	.060
6	4.2	.050
7	3.3	.042
8	2.1	.033
9	1.0	.028
10	1.1	.024
11	0.8	.020
12	0.6	.017
13	0.3	.0145
14	0.1	.0120
Σ	20.2	

$$\frac{\Sigma\,(N)(\text{Transmission})}{20.2} = 0.0475$$

FIG. 2. Example of the procedure used to calculate light input to each lake during winter, based on 20 biweekly measurements of snow depth per lake. The frequency of measurement occurrence at each centimeter of snow depth was plotted against that snow depth and a curve fitted by eye. The frequency of occurrence at each snow depth was then taken from the curve, multiplied by the light transmission at that snow thickness (from Fig. 1; albedo was automatically included), and the transmissions summed and divided by the sum of frequencies to give mean percent light transmission through the snow cover on that day.

at depth Z (I_Z) is approximated by the Beer–Lambert Law:

(1) $I_Z = I_o \cdot e - \epsilon^Z$

where I_o is the surface irradiance and ϵ is the extinction coefficient per meter. The extinction coefficient ϵ was estimated for each sample time as the slope of the linear portion of a log-linear plot of light at depth. Because of spectral differences in absorbance by water, extinction coefficients were sometimes higher in the uppermost 2 m, but we have ignored the effect.

To obtain daily, monthly, and annual lake-wide irradiance, continuous surface light in 5-min increments was applied against the appropriate albedo, snow, white ice, candled ice, black ice, and water transmittance factors to give irradiance at each metre of depth. Linear interpolation of factors was made between sampling dates.

Results and Discussion

Annual Cycle: Temperature

In midsummer (15 July–15 August), surface temperatures in small lakes reach 12–16°C, depending upon the year (Fig. 3). Weak stratification is often present, usually with a maximum range ≈3° between top and bottom, although a range of 8° was recorded on 22 July 1983, and 1 or 2 d of cold, windy weather is usually sufficient to break stratification and circulate the lake completely. Larger, deeper lakes such as Hawk and Turkey stratify more strongly, at least in some summers, than do the smaller lakes (Fig. 4). Epilimnion depth is greater at Saqvaqjuac than for similar lakes at lower latitudes (Patalas 1983), which we attribute to the higher mean wind speed, lack of wind protection by trees, and less intensive maximum solar radiation due to lower sun angle.

The lakes start cooling about 15 August and, with the advent of colder conditions in early September, cooling is very rapid, approaching $-1° \cdot d^{-1}$ ($\approx 330\ cal \cdot cm^{-2} \cdot d^{-1}$) in P & N Lake on cold, windy days. Although freezeup can occur anytime after the lakes drop below 4°C, the generally windy fall weather usually prevents icing over until the lakes are below 1°C, and often until they are isothermal at 0.1°C or less. Occasionally, such conditions have also been documented for moderate-sized temperate lakes (e.g. Lake Mendota, $Z_{mean} = 12.4$ m; Stewart 1973). Our observations show that lakes of 2 m mean depth freeze when the 5-d running mean air temperature reaches $\approx -3°C$, lakes of 3–4 m mean depth freeze at $\approx -5°C$, and lakes of 5 m mean depth freeze when the 5-d running mean reaches -6 to $-8°C$ (data not shown).

Immediately following freezeup and the sudden decrease in surface cooling, the water gains heat rapidly for several weeks (Fig. 3 and 5). Brewer (1958) ascribed a similar heating in Imikpuk Lake (Barrow, AK) to radiative heating, although he gave no data for solar radiation. We have calculated the heating of P & N Lake due to visible solar radiation (≈400–700 nm) in fall 1980. For the 17-d period 26 September (freezeup) to 12 October an average of 11.97 $E \cdot m^{-2} \cdot d^{-1}$ was received at the surface of which an average of 33% penetrated to the water surface after reflectance and attenuation through the slush ice and snow. Using a conversion factor of 20.9 $\mu\epsilon \cdot cal^{-1} \cdot cm^{-1}$ at 600 nm (Brock 1981), this was equival- ent to 18.9 $cal \cdot cm^{-2} \cdot d^{-1}$, very close to the 19.6 $cal \cdot cm^{-2} \cdot d^{-1}$ required to explain the rapid heat gain after freezeup (Fig. 5). We conclude therefore that radiative heating probably did account for the rapid rise in P & N Lake water temperature in late fall 1980.

By late October the mean water temperature is near 1.5°C, where it remains throughout the winter (Fig. 3). The heat content of the unfrozen lake decreases slowly as the ice thickens (Fig. 5). Apparently there is equilibrium between cooling at the ice–water interface (subice surface) and heating from the lake bottom. The slight warming of water adjacent to

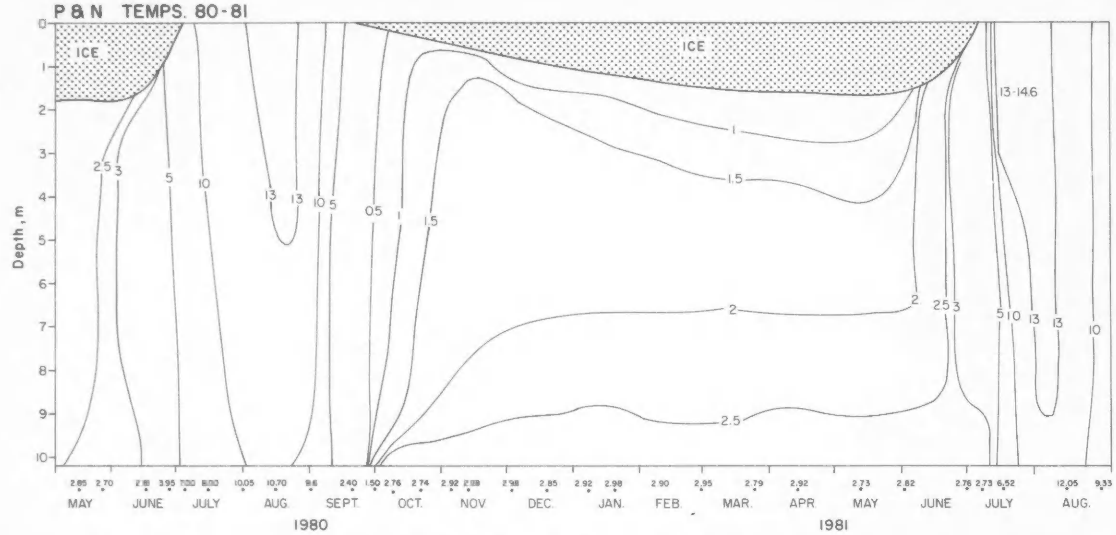


FIG. 3. Isotherms in P & N Lake, May 1980 -- September 1981. Maximum depth 10 m. Small numbers at the bottom of the graph are bottom mud temperatures on days when temperature profiles were made.

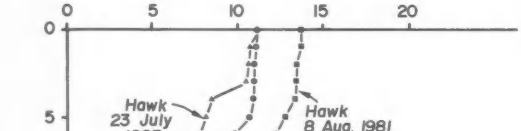


FIG. 4. Temperature profiles in Hawk and Turkey lakes in midsummer. BM = bottom mud temperature.

the bottom and the cooling at the subice surface result in density currents where warmer, higher salt content, denser water falls down slope and lighter, colder water moves shoreward just beneath the ice (Welch and Bergmann 1985).

The rate of ice accumulation depends upon air temperature but is modified as a negative function of snow cover and thus varies from lake to lake and year to year. For example, maximum ice thickness on P & N Lake averaged 2.0 m in 1978 whereas in 1981 it averaged only 1.65 m because of heavy snow cover (Fig. 6). In 1983, maximum ice thickness reached 2.4 m and averaged over 2.1 m on P & N Lake because of very low winter snow cover (data not shown).

Snow cover on lakes varies day to day because storms may result in either net deposition or net removal, although removal usually occurs only for relatively soft wind pack deposited in early winter or in the early stages of a storm. For example, in early December 1977 a storm of deposition increased snow cover on all lakes, but a storm of erosion later decreased the cover (Fig. 6). The straight lines connecting the points in Fig. 6 are actually incorrect, since sufficient data would show that snow changes occur in stepwise fashion as a function of storm occurrences.

The development of white ice (see Appendix) decreases with increasing latitude, being generally present on temperate lakes (e.g. Adams and Roulet 1980; Welch et al. 1976) but rare at 75°N (Schindler et al. 1974; H. E. Welch, pers. obs.). At Saqvaqjuac (≈64°N), white ice is formed on most small lakes during years of early heavy snowfall. In 1977−78, white ice averaged 4 cm on Far Lake, 6 cm on Methane North, and 11 cm on P & N Lake. In 1980−81 it averaged 6 cm on Jade Lake, which froze early (22 September) and had early heavy snow accumulation. Far and P & N Lakes had only a small patch of white ice averaging 6 cm at the lake centers as a result of sampling activities. Spring Lake also froze early in 1980, received a relatively heavy snow cover like Jade Lake, and developed a fairly uniform white ice cover. Most of the snow subsequently blew off in early October so that by the time we began routine snow measurements on 16 October there was less than 1 cm of snow (Fig. 6) but 5 cm of white ice remaining as a result of the earlier snow cover. Hawk and Turkey lakes had no white ice in 1980−81. Such larger lakes tend to freeze

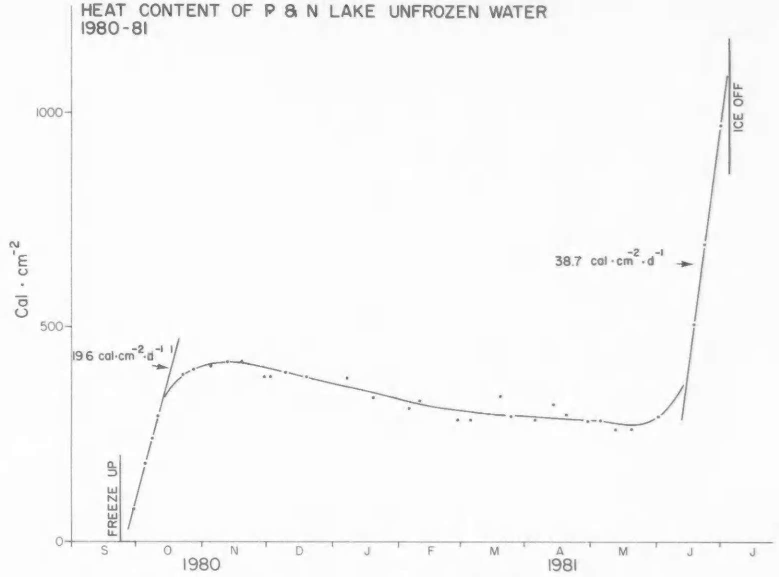


FIG. 5. Heat content of the unfrozen lake body during the winter of 1980–81.

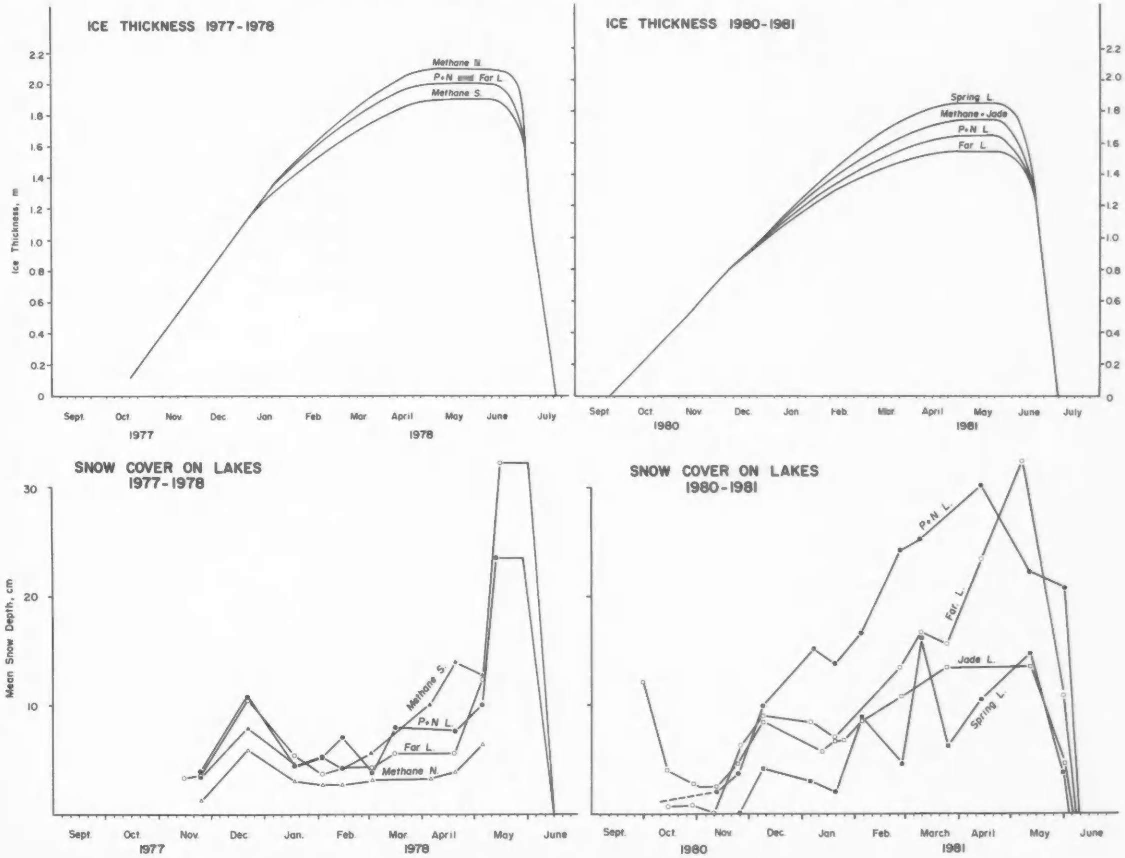


FIG. 6. Mean ice and snow thickness on Saqvaqjuac lakes.

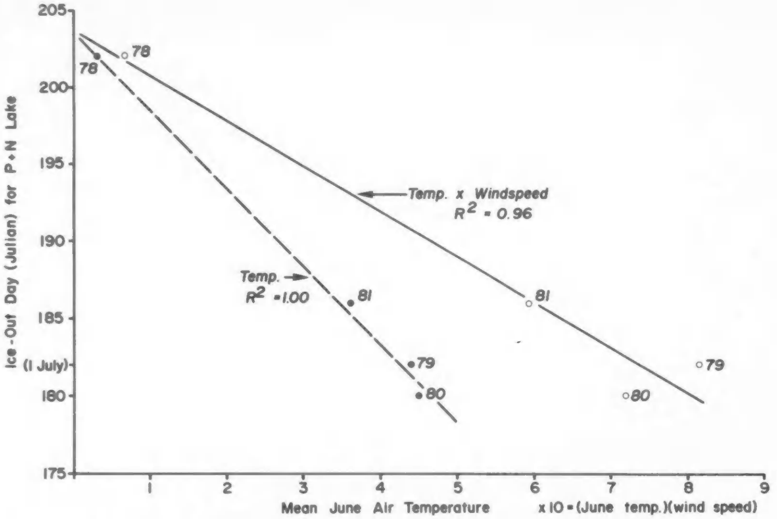


FIG. 7. Ice-out data for P & N Lake as a function of antecedent weather in June 1978–81.

later and develop initial ice cover faster, resulting in little or no white ice development most years. The biological significance of white ice lies in its light attenuation properties (see below).

Snow melt begins 25 May–5 June (Fig. 6; Welch and Legault 1986), and with the disappearance of snow cover on the lakes, heating is rapid (38.7 cal·cm^{-2}·d^{-1} in P & N Lake 1981, Fig. 5). The heating is usually rapid enough to produce temporary temperature, and presumably density, inversions in the bottom waters in mid- to late June (P & N Lake, Fig. 3). We have not seen significant temperature inversions immediately beneath the ice (Bergmann and Welch 1985; Fig. 1) such as La Perriere (1981) found in Harding Lake, AK (64°24′N). The inflowing meltwater and lake ice meltwater form a cohesive unit which leaves the lake mostly intact as subice flow (Bergmann and Welch 1985).

When the earliest meltwater first leaves the land and flows out over the ice, the ice tends to float, creating a positive hydraulic head for the surface water. As candling begins, the first vertical channels in the ice appear, apparently where bits of frozen debris absorb radiation and melt the surrounding ice first. The surface water then falls down these holes, enlarging them rapidly and often resulting in one to several shallow drainage channels radiating outward from the hole. Drainage through such holes is sufficiently strong to create whirlpools and suck pieces of ice several centimetres long under the water surface. These downward-flowing plumes of meltwater may reach the lake bottom several metres below, eroding flocculent surface sediments and leaving a 1–3 m "blowout" or light-colored patch which is readily visible through the hole after this early and rapid drainage is finished. However, because there are few of these drainage holes (typically one to three on a 5-ha lake) and because they are ephemeral, disappearing as soon as candling proceeds and the ice becomes generally porous, they do not result in large-scale vertical mixing during the main melt period (Bergmann and Welch 1985).

A moat forms around the edges of the lakes, and as melt progresses the central ice pan becomes free of shore. However, at 64°N, moat development and the freeing of a thick, central candled ice pan is not as striking as in the high arctic because melting is more rapid once it begins and the central pan is quite thin once it is freed from shore.

Ice-out at Saqvaqjuac ranges from about 28 June to 21 July in the small lakes, with very large lakes (>10 km^2) becoming ice-free about 10 d later. In 1978, Puuqzinjac, a 10-km^2 lake 10 km northeast of Saqvaqjuac Inlet, had a nearly complete ice cover on 1 August. Ice-out times for small Saqvaqjuac lakes are a function of temperature. Mean June temperature and, to a lesser degree, the product of mean June temperature times wind speed are good predictors of ice-out for small Saqvaqjuac lakes (Fig. 7).

We have calculated that for the 23-d period between 11 June 1981 when PN Lake ice surface became snow free, and ice-out on 3 July, a total of 869 E·m^{-2} surface PAR was received, of which 27.4% was reflected and 72.6% passed through the candled ice surface. Three hundred seventy-two Einsteins per square meter or 77 cal·cm^{-2}·d^{-1} entered the water, twice the 38.7 cal·cm^{-2} required to heat the water column (Fig. 5); the excess was probably transferred to cold inflowing meltwater and the subice surface. Two hundred fifty-nine Einsteins per square meter or 1237 cal·cm^{-2} was absorbed by the ice, only 10% of the 12 400 cal·cm^{-2} required for melt. Assuming that 869 E·m^{-2} total surface PAR, or $\approx$4158 cal·cm^{-2}, was half the total radiation, the total radiant energy flux per unit area was equal to about two thirds of the $\approx$13 400 cal·cm^{-2} required to melt the ice and raise the water temperature of P & N Lake, disregarding albedo. Convective heat transfer, readily obvious to an observer moving up and downwind of a Saqvaqjuac lake in June, therefore supplies a great deal of heat energy to the surfaces of melting lakes. We measured ice loss at 25 locations on P & N Lake in June 1981 and found that 63% of the first 60-cm decrease in ice thickness occurred at the surface. Considering that lakes occupy about 17% of the Saqvaqjuac land surface (Welch 1985), the local climatic effect of lakes must be considerable during spring melt.

Annual Cycle: Heat Budgets

Annual heat budgets of arctic lakes are similar in magnitude to those of low-latitude lakes, but much of the heat flux occurs

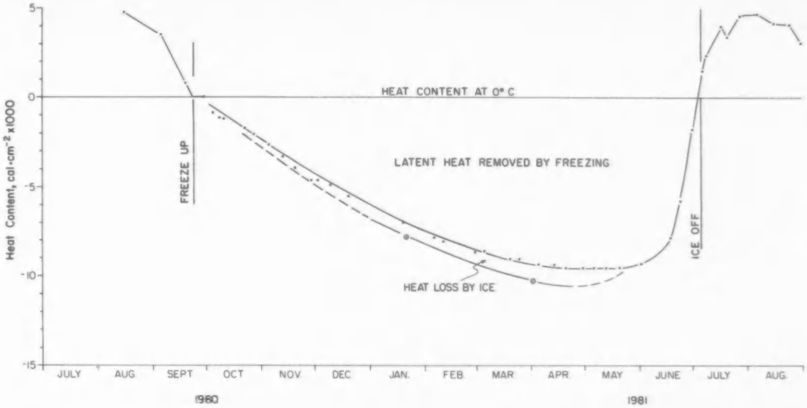


FIG. 8. Lake-wide mean heat content and flux for P & N Lake, 1980–81. Heat loss by ice is the heat content of the ice at 0°C minus the heat content assuming linear interpolation between the top ice surface at mean ambient air temperature and the bottom surface at 0°C.

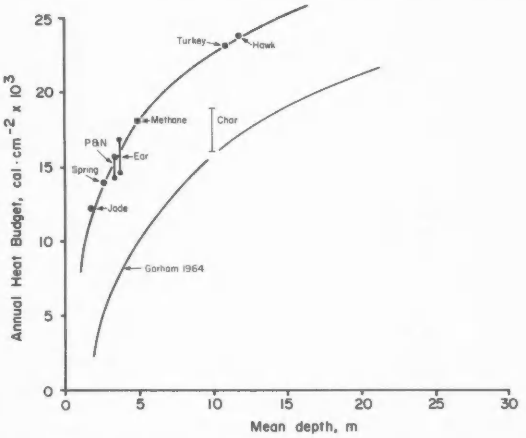


FIG. 9. Annual heat budget of Saqvaqjuac lakes as a function of mean depth.

as latent heat changes during freezing and thawing. For small lakes the heat budgets (maximum summer heat content plus maximum winter debit, including latent heat of fusion) is about two thirds latent heat of fusion (e.g. P & N Lake, Fig. 8). Lakes of mean depth ≥10 m have a higher summer heat content; Hawk Lake (Z_{mean} = 11.6 m) had a summer heat budget of 12 780 cal·cm^{-2} in July 1981, 54% of its total heat budget. The relationship between annual heat budget and mean depth for Saqvaqjuac lakes is closely approximated by a logarithmic function which does not asymptote (Fig. 9). Gorham (1964) also found that heat budgets of warm monomictic and dimicitic lakes (mean depths up to several hundred metres) did not asymptote with increasing depth (data mostly from Hutchinson 1957).

Although the heat budgets shown in Fig. 8 are comparable with others used in classical limnology (e.g. Wetzel 1975), three additional terms should be incorporated to provide a reasonably complete heat flux. The first is the heat used in heating the ice from its lowest temperature to its melting point (Strom 1944, quoted in Hutchinson 1957, p. 495). Assuming a linear interpolation between 0°C at the ice–water interface and −30°C at the upper ice surface, Fig. 8 shows that this "cold ice" term is only a small part of the heat budgets, amounting to some 920 cal·cm^{-2} on 1 April for P & N Lake, or an additional 6% to the P & N Lake heat budget for 1980–81. The second term that should be considered is the latent heat of vaporization. A mean of about 0.25 vertical m of water was retained (= evaporated) by P & N Lake for 3 yrs 1979–81 (Welch and Legault 1986). This is equal to about 13 400 cal·cm^{-2}, or nearly as much as the classical heat budget for P & N Lake (Fig. 8). The third term that is sometimes considered is heating of the lake bottom (Likens and Johnson 1969), which can be a high proportion of total summer heat income in shallow basins (Hecky 1984). We did not calculate bottom heating because of insufficient data.

The Saqvaqjuac heat budgets are higher than the lower-latitude budgets analyzed by Gorham. We expect that lakes a few hundred kilometres west of Saqvaqjuac have even higher annual heat budgets becasue the continental climate and low snow fall on the central arctic barrens should result in maximum summer heating combined with maximum winter ice development. The budget of Char Lake (74°56′N; Z_{mean} = 10.2 m) falls below the Saqvaqjuac regression line in Fig. 9 but is still above the mean for temperate lakes of comparable size. However, since evaporation from low-latitude lakes must be much higher than for arctic lakes, the addition of an evaporation term would probably increase the calculated heat income for low-latitude lakes to levels at or higher than that for Saqvaqjuac lakes.

Annual Cycle: Light

Extinction coefficients for P & N , Far, Spring, and Methane lakes ranged from 0.25 to 0.76 during June–September, excluding fertilizatoin years when extinction coefficient tended to be higher in P & N, Jade and Far lakes. Mean annual extinction coefficients were inversely correlated with mean depth (Fig. 10). Fertilization apparently resulted in increased turbidity as phytoplankton increased (P & N and Jade lakes, Fig. 10). But Far Lake, whose phytoplankton levels did not increase significantly after phosphorous-only fertilization

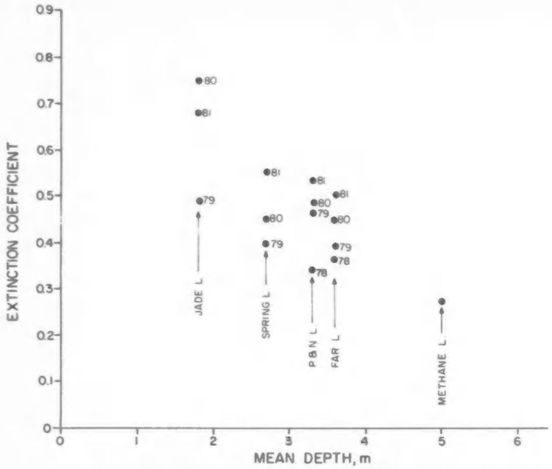


FIG. 10. Extinction coefficients for Saqvaqjuac lakes, mean for June – September. Methane Lake = 1977; Far Lake = 1977–78 prefertilization 1979–81, phosphorus addition; P & N Lake = 1977–78 prefertilization 1979–81 phosphorus and nitrogen addition; Spring Lake = 1979–81. Jade Lake = 1979–80 phosphorus and nitrogen addition and 1981 postfertilization.

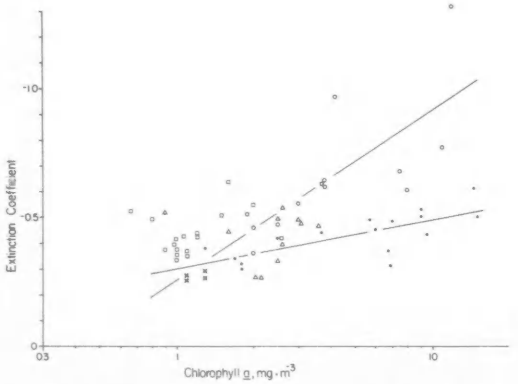


FIG. 11. Extinction coefficient as a function of chlorophyll a concentration in Saqvaqjuac lakes. ●, P & N Lake; ○, Jade Lake; △, Spring Lake; □, Far Lake; ×, Methane Lake. For Jade Lake, extinction coefficient = 0.253 + 0.288 Chl a (r^2 = 0.54); for P & N Lake, extinction coefficient = 0.300 + 0.081 Chl a (r^2 = 0.53).

(unpublished), and Spring Lake (control) also showed increases in light extinction during 1979–81 (Fig. 10).

We have plotted light extinction coefficients as a function of chlorophyll concentration in Fig. 11. For Jade and P & N lakes, which had ≈10-fold range in chlorophyll, correlation coefficients were 0.54 and 0.53 respectively, although the data sets do not overlap on the high-chlorophyll end. Data for three other lakes are clustered in too narrow a range to show much, although at a given chlorophyll concentration it appears that each lake has a characteristic range of light extinction which is being influenced by factors other than chlorophyll, one of which is mean depth (above). Chlorophyll concentration is therefore not a very good general predictor of light extinction in Saqvaqjuac lakes.

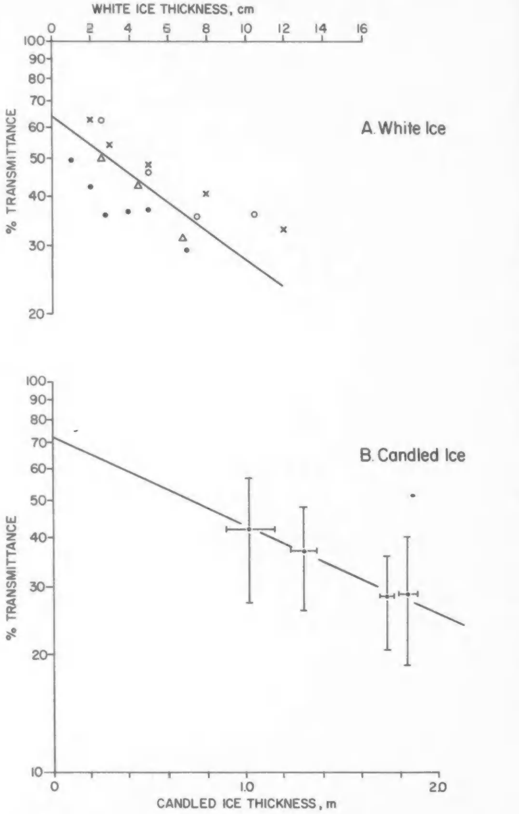


FIG. 12. Transmission of surface light through white and candled ice at Saqvaqjuac. For white ice, ln (transparency) = $0.0827x - 0.4503$ where x = white ice thickness, transmittance = $0.0794 \cdot \mathrm{cm}^{-1}$, and slope and intercept are means of four regression coefficients calculated from three to six thickness/transparency measurements made on four white ice sheets: ● and ×, P & N Lake; ○ and △, Far Lake. For candled ice, ln (transparency) = $-0.5229x - 0.3203$ where x = candled ice thickness and transmittance = $0.5928 \cdot \mathrm{m}^{-1}$. Date and number of data used to calculate transmission were 9 June (N = 69), 13 June (N = 69), 21 June (N = 85), and 26 June (N = 84). Ice thickness was the mean at seven locations on each date (see Methods for details). Error bars are ±2 SD.

Only one set of measurements of light transmission through snow was made at Saqvaqjuac (open circles in Fig. 1), which differs slightly from the light transmission reported by Welch and Kalff (1974) at 74°56′N through typical drifted snowpack (also shown in Fig 1). We used the "Saqvaqjuac" curve in Fig. 1 to calculate light transmission into Saqvaqjuac lakes rather than the "Resolute" curve because, despite the fewer data points, we think there is probably a slight but real increase in average light transmission at 64 compared with 74°N, since the high arctic snowpack seems slightly harder and denser. The two curves are identical at snow depths ≤5 cm, and the albedo is 60%. We recognize that these empirical snow transparency estimates are not rigorous from a physicist's point of view (e.g. Giddings and La Chapelle 1961; Warren 1982), but from a biological perspective they are probably adequate for calculation of phytoplankton photosynthesis.

White ice had an albedo of 36% (antiln −0.4503 = 0.64

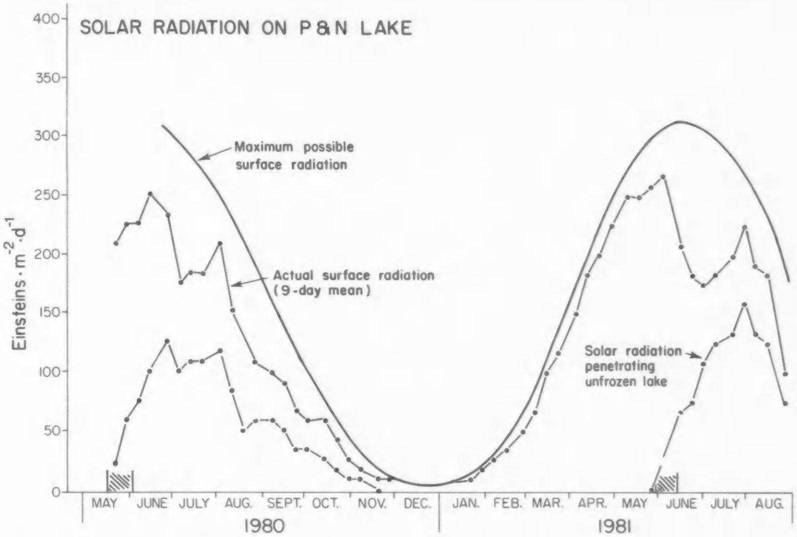


FIG. 13. Solar radiation (PAR, 400–700 mm) reaching the surface and penetrating the unfrozen water of P & N Lake, 1980–81 (typical for the research lakes at Saqvaqjuac). Hatched area shows the period of snowmelt for the two years.

transmission at 0 cm, Fig. 12), much lower than that of snow, but it was also relatively opaque. Ten centimetres of white ice transmitted 28% of incident light and 44% of absorbed light. The albedo of candled ice was only 27% (Fig. 12), with 1 m of candled ice passing 43% of incident light, or 59% of absorbed light.

The net results of various light factors are seen in Fig. 13. Actual solar radiation received at the land surface is close to theoretical maximum from midwinter to late May, after which time increasing cloud cover reduces the percentage reaching the surface. The effect is similar to but not quite as pronounced as that reported by Schindler et al. (1974) at Resolute (74°56′N). On high snow years (e.g. 1980–81, Fig. 3), very little light reaches the ice surface before spring melt, and any white ice present reduces transmission further. The percentage of incident light penetrating the water column therefore changes rapidly during spring melt (the hatched time window in Fig. 13), with peak radiation entering the lake at ice-out sometime in July. The steady decrease in water column irradiance during August–November parallels the decrease in surface irradiance caused by decreasing sun angle.

Snow (and to a much less extent, ice) greatly reduces the annual PAR input into lakes. At Saqvaqjuac we found that the mean of 110 open-water determinations during 1977–81 gave an average albedo of 32%, or 68% of incident PAR absorbed by the ice-free lakes in summer. But because of the ice and snow cover, only 34% of the annual total surface PAR penetrated to liquid water, and only 23% reaches the water in Char Lake (74°56′N) (H. E. Welch, unpubl.). Compared with the annual surface light that would be absorbed under totally ice-free conditions, therefore, only 34/68 = 50% reaches the water column at Saqvaqjuac, and only 23/68 = 34% penetrates to water in Char Lake. Annual primary production should show a similar gradient with latitude.

Adams et al. (1984) measured the spectral distribution of light penetrating an ice- and snow-covered lake (≈43°50′N) in February and found weak negative correlations between extinction coefficients and snow depth. But because light extinction in homogeneous snowpacks decreases asymptotically with depth (Fig. 1; Warren 1982) , we would expect either no correlation or, at low snow depths, a positive correlation between snow depth and extinction coefficient. The results of Adams et al. (1984) were likely artifacts of the measurement technique. They measured downwelling light through the composite snow and ice cover, then removed the overlying snow and again measured light at the subice surface, and attributed the difference to the snow. Because light was scattered by the ice cover beneath the snow, especially by opalescent white ice, the light flux below areas of high snow cover experienced a net increase from adjacent areas of low snow cover whereas low-snow areas had a net decrease. Thus, when subice light was measured before and after snow removal, there was a greater relative change per centimetre of snow in low-snow areas than in high-snow areas, resulting in apparently higher extinction in low-snow areas. This is clearly demonstrated by a comparison of our data in Fig. 1 and 14. Light transmission through snow plus ≈2.2 m of clear ice showed an apparently lower extinction (Fig. 13; ≈ −0.097 at snow >10 cm) than light transmission through the snowpack alone under similar conditions (Fig. 1; ≈ −0.165 at snow >10 cm) because of net flux into areas below snow drifts in the lake.

Compared with the enormous number and area of lakes in northern Canada, very little work has been done on their heat and light budgets. In areas such as Saqvaqjuac where lakes are an appreciable fraction of the land surface, lakes must ameliorate climatic extremes, especially in summer. Lake ice should be a sensitive climate indicator because ice-out time is very sensitive to antecedent weather (e.g. Fig. 7), and maximum winter ice thickness integrates winter temperature and snowfall. A series of lakes distributed along a latitudinal gradient might be a useful monitor of the climatic warming projected for arctic Canada (e.g. Manabe and Wetherald 1986).

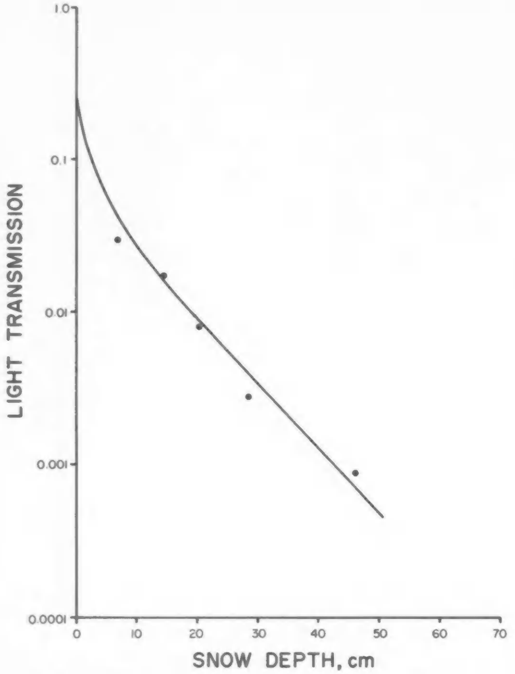


FIG. 14. Light transmission through wind-deposited arctic snowpack plus ≈2.2 m of ice (from Welch and Kalff 1974; data from Resolute, N.W.T.). Average light extinction coefficient below 10 cm snow depth ≈ −0.097.

The most important biological effects of ice and snow on lake biota undoubtedly result from the reduction of light flux into the lake. If photosynthesis is proportional to light input, then we would expect Saqvaqjuac lakes to be about one half and Char Lake to be about one third as productive as equivalent lakes much further south which do not freeze. Although this coincides with what is generally known about phytoplankton production in arctic lakes (e.g. Brylinsky and Mann 1973), if benthic photosynthesis is considered, photosynthesis is constant over a wide (20°) latitudinal gradient (H. E. Welch et al., unpubl.). In any case, not nearly enough attention has been paid to modelling light penetration through snow and ice, partly because physicists shy away from such heterogeneous material, and partly because biologists have not had primary production models sophisticated enough to require detailed winter light models until recently. Our candled ice extinction coefficient (Fig. 12) is probably widely applicable. The white ice coefficient (Fig. 11) is inherently variable because of the heterogeneous nature of white ice formation, and many more measurements in different situations will be required before reliable predictions can be made. Light attenuation by snow is quite predictable (Fig. 1) but requires refinement; measurements should be made which are functions of objective physical characteristics such as snow density, grain size, and compressibility. Better models of light penetration through snow (and sea ice) will be even more valuable for prediction of photosynthesis in arctic seas rather than lakes.

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Appendix

Clear freshwater ice is called "black" ice (being transparent, it appears colorless and hence very dark over deep water). Black ice generally has various horizontal layers of vertically elongate gas bubbles enclosed, but black ice on arctic lakes is usually almost bubble-free and appears as clear as glass. Slush or white ice is a composite of snow and lake water, formed when snow load depresses the ice surface below water level, lake water seeps through cracks and saturates lower snow layers, and the mass then freezes on top of previously formed black ice. White ice is somewhat transparent but images cannot be seen through more than 1–2 cm because of internal reflectance and scattering. The ice begins to candle in spring at the onset of melt, as thin drainage channels develop vertically from top to bottom in the ice sheet. The ice eventually becomes a mass of sharply pointed vertical shards (candles) whose transparency is high but with high internal reflectance and hence image distortion. In contrast with the sharp upper surface relief, the bottom surface of candled ice is as smooth as glass (at least in arctic lakes), perforated by the exposed ends of the millimetre-sized ribbon-like channels. Candles are loosely held together, and exposed ice edges over 1 m thick gradually disintegrate as candles fall away.

Photoperiod Control of Parr–Smolt Transformation in Atlantic Salmon (*Salmo salar*): Changes in Salinity Tolerance, Gill Na$^+$,K$^+$-ATPase Activity, and Plasma Thyroid Hormones

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Atlantic salmon (*Salmo salar*) were subjected to artificial photoperiods to determine the manner and extent of photoperiod control of the parr–smolt transformation. Exposure to continuous light (L24) at first feeding and maintained throughout the rearing period inhibited increases in salinity tolerance and gill Na$^+$,K$^+$-ATPase activity that occurred in spring in fish raised under simulated natural photoperiod (SNP). Fish reared under continuous light and returned to SNP in October (L24OCT) underwent normal increases in salinity tolerance and gill Na$^+$,K$^+$-ATPase activity, whereas those returned in December (L24DEC) underwent delayed and intermediate increases. Plasma thyroxine peaks occurred simultaneously in all groups but were diminished in the L24 and L24DEC groups. Plasma 3,5,3′-triiodo-L-thyronine levels were not affected by any photoperiod treatment. Inhibition of the parr–smolt transformation decreased the potential for growth in seawater. In spite of changes in the timing of the transformation induced by photoperiod treatment, salinity tolerance and gill Na$^+$,K$^+$-ATPase activity were strongly correlated; correlation between changes in salinity tolerance and plasma thyroid hormones were, by comparison, weak. The results demonstrate that continuous light applied early in ontogeny and maintained throughout the rearing period inhibits osmoregulatory changes associated with parr–smolt transformation, whereas increasing day length during winter–spring stimulates transformation.

On a soumis des saumons de l'Atlantique (*Salmo salar*) à des photopériodes artificielles afin de déterminer comment et dans quelle mesure la photopériode régit la transformation d'un tacon en smolt. L'exposition continue à la lumière (L24) au moment de l'alimentation initiale et pendant toute la période de croissance a bloqué l'augmentation de la tolérance à la salinité et de l'activité du Na$^+$,K$^+$-ATPase dans les ouïes qui est observée au printemps chez des poissons soumis à une photopériode naturelle simulée (PNS). Chez les poissons gardés en milieu continuellement éclairé et exposés à une PNS en octobre (L24OCT), on a observé des augmentations normales de la tolérance à la salinité et de l'activité du Na$^+$,K$^+$-ATPase dans les ouïes; par contre, les poissons exposés à une PNS en décembre (L24DÉC) ont montré des augmentations intermédiaires et retardées. Les teneurs en thyroxine plasmatique ont atteint des pointes simultanément chez tous les groupes mais étaient réduites chez les groupes L24 et L24DÉC. Les teneurs en 3,5,3′-triiodo-L-thyronine plasmatique n'ont pas été touchées par les modifications de la photopériode. L'inhibition de la transformation tacon–smolt a réduit le potentiel de croissance en milieu marin. Malgré des variations du moment de la transformation induites par la photopériode, la tolérance à la salinité et l'activité du N$^+$,K$^+$-ATPase dans les ouïes étaient fortement corrélées. En comparaison, la corrélation entre les variations de la tolérance à la salinité et les hormones thyroïdiennes plasmatiques était faible. Les résultats révèlent qu'une illumination continue appliquée au début de l'ontogenèse et maintenue pendant toute la période de croissance paralyse les changements osmorégulatoires liés à la transformation tacon–smolt tandis qu'une augmentation de la longueur de la journée en hiver et au printemps stimule la transformation.

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The life history of anadromous Atlantic salmon (*Salmo salar*) is characterized by a rapid, synchronous seaward migration of juveniles in spring. The migrant juvenile, known as a smolt, can be distinguished from the riverine parr by its larger size, absence of vertical pigmented bars (parr marks), blackening of the paired and caudal fins, and intense silvering of the skin and scales which is the result of guanine and hypoxanthine deposition (Johnston and Eales 1967; Wedemeyer et al. 1980). The parr–smolt transformation is characterized by a number of changes in osmoregulatory physiology that occur in freshwater and result in increased salinity tolerance (see reviews by Hoar 1976; McCormick and Saunders 1987). Prominent among these preparatory physiological adaptations are increases in the activity of gill Na$^+$,K$^+$-ATPase, an enzyme which plays a central role in active ion transport (Maetz and Garcia-Romeau 1964; Silva et al. 1977). Circulating levels of thyroid hormones also increase during transformation (Dickhoff et al. 1978, 1982), though their role with regard to osmoregulatory changes has yet to be established.

Several studies have implicated photoperiod in controlling the timing of the parr–smolt transformation in Atlantic salmon and other anadromous salmonids (Saunders and Henderson

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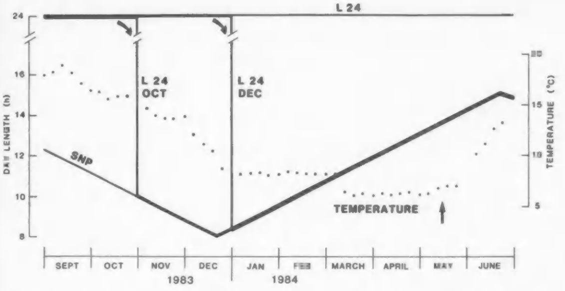

FIG. 1. Changes in day length and temperature during experimental photoperiod treatment. Vertical arrow indicates time at which fish were transferred to sea cages.

1970; Zaugg and Wagner 1973; Wagner 1974a; Komourdjian et al. 1976; Clarke et al. 1978, 1985; Saunders et al. 1985). The nature of this control has not been established, nor has a practical method for the temporal manipulation of the parr–smolt transformation been developed. Saunders et al. (1985) found that exposure of juvenile Atlantic salmon to continuous light in autumn increased growth rate but had a deleterious effect on salinity tolerance in spring and inhibited other aspects of the transformation. The present study was initiated to determine how and to what extent photoperiod controls the parr–smolt transformation. We also hoped that environmental manipulation of the transformation would shed light on temporal changes in salinity tolerance, gill Na^+,K^+-ATPase activity, and thyroid hormone levels.

Materials and Methods

Experiment I

Atlantic salmon of mixed river stock were incubated and hatched at the North American Salmon Research Center and brought to the St. Andrews Biological Station just prior to first feeding. They were reared in 1-m-diameter tanks supplied with freshwater at 12 $L \cdot min^{-1}$. Coincident with first feeding (May 25, 1983), fish were divided into four groups of approximately 1000 fish (two replicate tanks per group). Three of the groups were exposed to continuous light (24 $h \cdot d^{-1}$) and the remaining group was subjected to simulated natural photoperiod (SNP). Standard fluorescent bulbs provided light intensities at the water surface of 430–540 lx. On October 31, one group (L24OCT) was returned to SNP (Fig. 1). On December 31, a second group (L24DEC) was returned to SNP. A third group remained under continuous light (L24). Water temperature fluctuated seasonally (range 6–18°C) except during the period of physiological testing (January–May 1984) when it was relatively constant (6–8°C). During the laboratory phase of the study (May 1983–May 1984), salmon were fed commercial dry pellets (EWOS) to satiation (adjusted for temperature and body size (Saunders et al. 1985)) at half-hour intervals during daylight hours of the SNP group.

On December 21, fish smaller than 12.5 cm were removed from each group (approximately 25% of the fish in each tank) so as to reduce crowding and provide fish sufficiently large to undergo smoltification. Fish smaller than 10.3 cm (reared previously under SNP) were placed in a separate tank at the same thermal and photoperiod regimes as the SNP group and were designated the PARR group.

At monthly intervals, 50% of the fish in each tank were sampled randomly for measurement of length and weight. The fish were anaesthetized (1% tert-amyl alcohol), weighed to the nearest 0.1 g, and measured for fork length to the nearest 0.1 cm. No mortalities resulted from this procedure, and fish resumed feeding within hours.

Monthly between January and March and biweekly from April through May, 8 salmon from each group were killed for blood and tissue sampling, and 10 fish per group were subjected to high salinity. Salinity tolerance was measured by direct transfer of fish to 40‰ seawater for a period of at least 96 h as described by Saunders et al. (1985). For blood and gill sampling, fish were starved overnight prior to sampling between 12 30 and 15 00 Atlantic Standard Time. After measuring length and weight, anaesthetized fish were blotted dry, the caudal peduncle was severed, and blood was collected in two heparinized microhematocrit tubes which were centrifuged for 5 min at 5500 rpm. Plasma was removed and stored at −17°C. Gill arches were removed immediately after blood collection as described below.

Over a 2-wk period beginning May 11, 250–350 fish from each group (except PARR) were gradually acclimated to 30‰ seawater. On May 22 they were given pelvic and/or adipose fin clips to distinguish the four groups and were transported to a commercial salmon farm at Grand Manan Island, New Brunswick. The four groups were placed in a 20-m^2 floating net enclosure. Fish were fed moist pellets containing ground herring and groundfish supplemented with vitamins. Between May and November, temperature and salinity ranged from 6 to 12°C and 28 to 30‰. On October 11, 1984, at least 60 fish per group were anaesthetized and measured for length and weight. On November 8 and 25, blood and gills were sampled as described above with the exception that blood was sampled from the dorsal aorta with a heparinized syringe.

Analytical Techniques

Primary gill filaments (0.05–0.20 g wet weight) were trimmed from gill arches (ceratobranchials), placed in 1.0 mL of SEI buffer (0.3 *M* sucrose, 0.02 *M* disodium ethylenediaminetetraacetate, and 0.1 *M* imidazole at pH 7.1) and stored for 1–4 wk at −80°C. Gill Na^+,K^+-ATPase activity was determined by the method of Zaugg (1982) with the following modifications. Gill homogenates were incubated in 115 m*M* NaCl, 55 m*M* KCl, 17 m*M* $MgCl_2$, and 85 m*M* imidazole at 37°C and pH 7.0. Inorganic phosphate was determined by the method of Heinonen and Lahti (1981). Protein determination was performed by the method of Miller (1959) using bovine serum albumin as standard. Gill Na^+,K^+-ATPase activity was expressed as micromoles inorganic phosphate per milligram protein per hour. Intra- and interassay coefficients of variation of pooled and separated gill samples were 3.3% ($n = 4$) and 11.8% ($n = 4$) with two to four replicates each, respectively.

Plasma samples were stored for up to 6 mo at −17°C. Thyroxine (T4) and 3,5,3′-triiodo-L-thyronine (T3) concentrations were measured by radioimmunoassay (Dickhoff et al. 1978; McCormick and Naiman 1984). Charcoal-stripped Atlantic salmon plasma was used to make all standards. Intra- and interassay coefficients of variation for these assays were 5–13 and 9–12%, respectively. Plasma osmolarity was measured with a Wescor vapor pressure osmometer; all samples

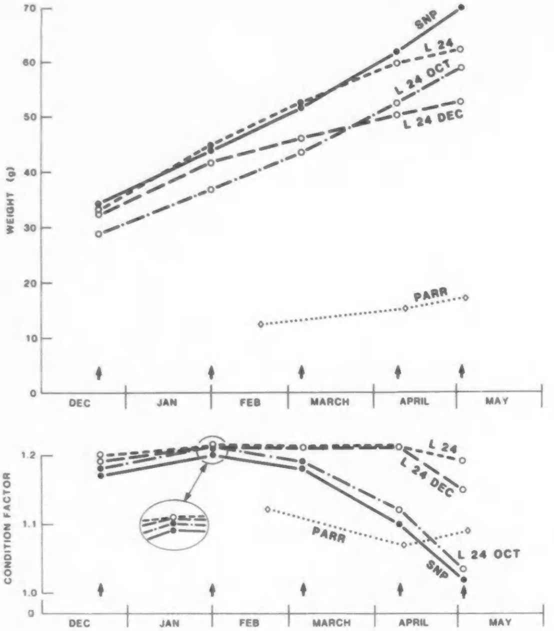

FIG. 2. Weight and condition factor of Atlantic salmon subjected to various photoperiod regimes. Values are the mean of 200–250 fish per group. Standard errors were less than 1% of the mean in each group (contained within the symbol for each mean). Significant changes in weight and condition factor over time occurred in each group ($p < 0.001$, one-way ANOVA). Open symbols represent a significant difference from the SNP group at a given time interval ($p < 0.05$, one-way ANOVA, Student–Newman–Keuls procedure).

were measured in one assay in which the intraassay coefficient of variation was 0.8% ($n = 5$).

Statistical comparisons of photoperiod treatment effects were performed with two-way analysis of variance. Interactions between photoperiod treatment and time were not significant. Differences among treatment groups at any time and changes over time within each treatment group were compared by Student–Newman–Keuls procedure. Statistical analysis of proportions (percent survival) was by Brownlee's test (Brownlee 1960). A significance level of $p < 0.05$ was used to establish statistically significant differences. All values are reported as mean and standard error (SE) unless otherwise stated. Condition factor is defined as ((weight/length^3)·100) with weight expressed in grams and length in centimetres.

Results

Experiment 1

Photoperiod and growth

In mid-December, fish in each group (except PARR) that were smaller than 12.5 cm were removed from the study so as to provide fish large enough to undergo transformation. After grading, the L24OCT group was 20% smaller ($p < 0.05$) than the SNP group (indicating a lower growth rate in the period prior to grading), but grew at a similar rate thereafter (Fig. 2).

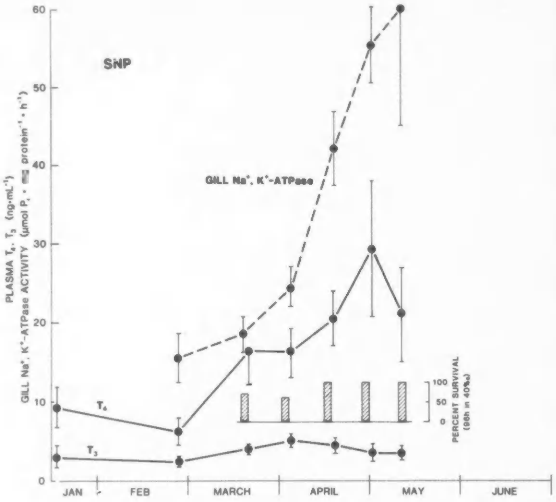

FIG. 3. Changes in gill Na^+,K^+-ATPase activity, plasma thyroid hormones, and salinity tolerance (percent survival for 96 h in 40‰ seawater) of Atlantic salmon exposed to SNP. Values are the mean ± 95% confidence interval of 8–10 fish at each sampling interval.

Beginning in mid-March and for the remainder of the study, the rate of change in weight of the L24DEC group was lower than that of the SNP and L24 groups. In each of these cases, a decline in day length (change from continuous light to simulated natural photoperiod) resulted in lower growth rates relative to SNP and L24 fish. In the case of the L24OCT group, this decline in growth rate was of short duration. L24 and SNP fish were of similar size (within 5% of one another) until the last weighing interval (early May).

Condition factor was similar in all photoperiod groups until the April–May period, when substantial decreases (>15%) occurred in both the SNP and L24OCT groups (Fig. 2). A slight decrease also occurred in the L24DEC group in May, while the L24 group remained nearly constant throughout the experiment. Condition factor of the PARR group, which in February was lower than the other groups, remained relatively constant between February and May.

Photoperiod and physiological change during parr–smolt transformation

Significant changes in salinity tolerance, gill Na^+,K^+-ATPase activity, and plasma thyroid hormones occurred between January and May under SNP (Fig. 3). Salinity tolerance had already reached 70% survival after 96 h in 40‰ seawater by mid-March, reached a maximum of 100% by mid-April, and remained at 100% through mid-May. Gill Na^+,K^+-ATPase activity rose steadily from late February to mid-May, increasing by 300% over this period. Plasma T4 also rose from a low mean value of 6.3 ng·mL^{-1} in late February to a peak of 29.5 ng·mL^{-1} in early May and subsequently declined to 7.1 ng·mL^{-1} in mid-May. Absolute and relative changes in plasma T3 were smaller than changes in plasma T4, though significant increases did occur. Plasma T3 rose from a low mean value of 2.6 ng·mL^{-1} in late February to a peak of 5.2 ng·mL^{-1} in early April and subsequently declined to 3.5 ng·mL^{-1} in early and mid-May.

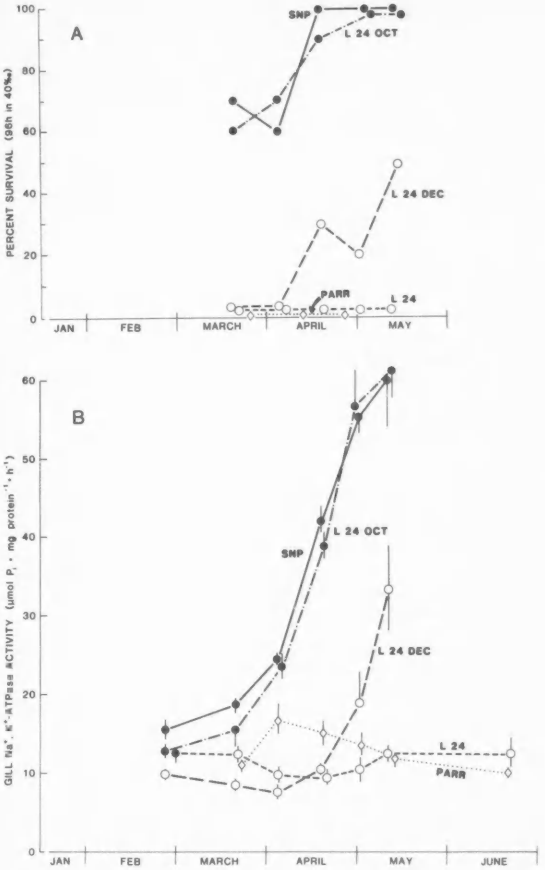

FIG. 4. (A) Percent survival after 96 h in 40‰ seawater of Atlantic salmon exposed to photoperiod treatment. Ten fish per group were used at each time interval. Open symbols represent a significant difference from the SNP group at a given time interval ($p < 0.05$, Brownlee's test). (B) Gill Na$^+$,K$^+$-ATPase activity of Atlantic salmon exposed to photoperiod treatments. Eight fish per group were used at each time interval. There was a significant effect of photoperiod treatment and date of sampling on gill Na$^+$,K$^+$-ATPase activity ($p < 0.05$, two-way ANOVA). Significant changes over time occurred in all groups except L24 ($p = 0.05$, one-way ANOVA). Open symbols represent a significant difference from the SNP group at a given interval ($p < 0.05$, one-way ANOVA, Student–Newman–Keuls procedure).

Salinity tolerance levels in both the SNP and L24OCT groups rose from 60–70% in mid-March to 100% by early May and were not significantly different from one another at any time (Fig. 4). No fish in either the L24 or PARR group was able to survive as long as 96 h in 40‰ seawater at any time between March and May. Salinity tolerance in the L24DEC group was 0% through early April, but increased to 50% by mid-May, indicating a delayed and perhaps diminished development of salinity tolerance in this group relative to the SNP group.

Changes in gill Na$^+$,K$^+$-ATPase activity in response to photoperiod treatment (Fig. 4) were similar to those for salinity tolerance. Gill Na$^+$,K$^+$-ATPase activity levels increased steadily between late February and mid-May in the SNP and L24OCT groups and were not significantly different from one another at any time. Gill Na$^+$,K$^+$-ATPase activity of the L24 group was not significantly different from the SNP and L24OCT groups in late February, but did not increase significantly throughout the freshwater sampling period ending in late June. A significant increase did occur in the PARR group between mid-March and mid-April, but this increase was slight (40%) relative to the 300% increase in the SNP and L24OCT groups. Gill Na$^+$,K$^+$-ATPase activity of the L24DEC group was significantly lower than the SNP group at all times, but increased by 200% between early April and mid-May, again demonstrating delayed development. Significant correlation ($r^2 = 0.84$) existed between the development of gill Na$^+$,K$^+$-ATPase activity and salinity tolerance (Fig. 5).

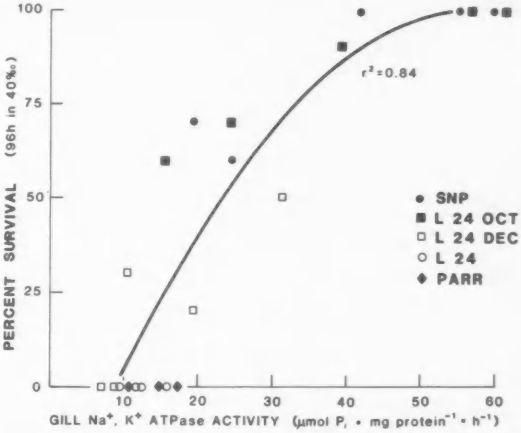

FIG. 5. Correlation between salinity tolerance and gill Na$^+$,K$^+$-ATPase activity for all photoperiod treatments at each sampling period. Each point represents the mean values reported in Fig. 4 and 5. Regression curve was fitted using a second-order polynominal with the following equation: $y = -0.042x^2 + 4.9x - 41.1$.

Plasma T4 levels increased in all groups in spring (Fig. 6). Highest mean plasma T4 levels were found consistently in the SNP group. Differences in plasma T4 can be most easily compared among groups by examining early May values when plasma T4 (as well as salinity tolerance and gill Na$^+$,K$^+$-ATPase activity) in the SNP group was at its peak. At this time, plasma T4 levels in the L24OCT group were not significantly different from SNP, while L24DEC, L24, and PARR had significantly lower levels. However, whereas a developmental shift occurred in the timing of increases in salinity tolerance and gill Na$^+$,K$^+$-ATPase activity of the L24DEC group, no such temporal shift occurred in their plasma T4 levels. In addition, whereas no salinity tolerance developed in the L24 and PARR groups, there were significant increases in plasma T4 in these two groups (albeit to a lesser degree and for the PARR group at a later time).

Plasma T3 levels were not significantly affected by photoperiod treatment (excluding the PARR group; Fig. 6). With the exception of lower levels in the PARR group, no consistent differences in plasma T3 were observed among the groups. The absence of concurrent changes in plasma T3 and other physiological changes is exemplified by comparison of the

TABLE 1. Length, growth, and osmoregulatory parameters of Atlantic salmon (subjected to prior photoperiod treatment) after 5 mo in sea cages. Values are expressed as mean (SE); length in cm, growth rate in $mm \cdot d^{-1}$, osmolarity in mosm/L, gill Na^+,K^+-ATPase activity in $\mu mol\ P_i \cdot mg\ protein^{-1} \cdot h^{-1}$, and thyroid hormones in $ng \cdot mL^{-1}$. Significant differences ($p < 0.02$) in plasma thyroid hormone levels were found among prior photoperiod treatments; groups with different letter designations were significantly different from one another ($p < 0.05$, Student–Newmand–Keuls procedure).

Prior photoperiod treatment	Initial length (May) (n = 350–400)	Final length (October) (n = 60–66)	Growth rate	Plasma osmolarity (n = 8)	Gill Na^+,K^+-ATPase activity (n = 6)	Plasma T3 (n = 8)	Plasma T4 (n = 8)
SNP	18.9 (0.05)	35.1 (0.3)	1.0	339 (3.5)	29.4 (3.7)	6.1^a (1.0)	10.2^a (1.6)
L24OCT	17.8 (0.05)	33.0 (0.2)	0.9	345 (2.9)	—	3.1^b (0.5)	9.6^{ab} (2.3)
L24DEC	16.5 (0.05)	30.0 (0.3)	0.8	341 (4.2)	—	3.6^b (1.0)	5.6^{bc} (1.1)
L24	17.2 (0.06)	23.2 (0.5)	0.4	335 (4.0)	33.9 (3.9)	1.6^b (0.5)	4.3^c (0.7)

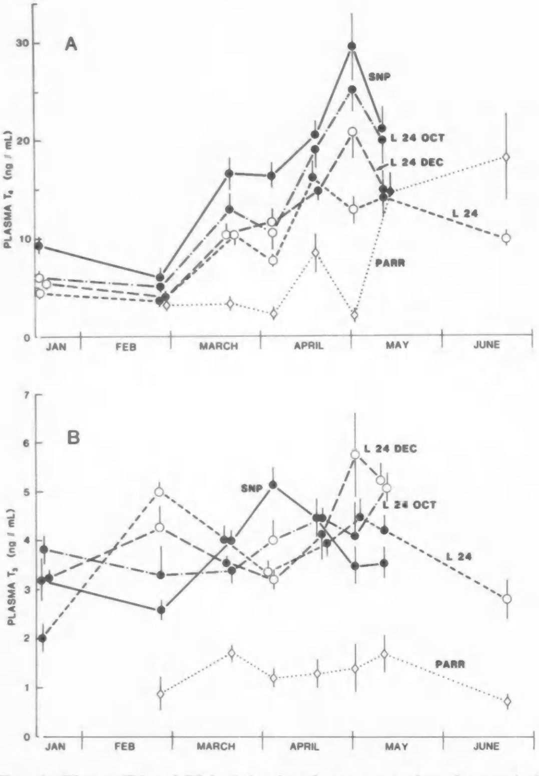

FIG. 6. Plasma T4 and T3 in Atlantic salmon exposed to photoperiod treatments. Eight fish per group were used at each time interval. There was a significant effect of photoperiod treatments and date of sampling on plasma T4 ($p < 0.05$, two-way ANOVA), but no significant effect of photoperiod treatment (excluding the PARR group) on plasma T3 ($p > 0.2$). Significant changes over time occurred in plasma T4 and T3 in all groups, except for plasma T3 in the L24OCT and PARR groups. Open symbols represent a significant difference from the SNP group at a given time interval ($p < 0.05$, one-way ANOVA, Student–Newman–Keuls procedure).

SNP and L24OCT groups which had identical patterns of increase in salinity tolerance and gill Na^+,K^+-ATPase activity but which were dissimilar in their plasma T3 changes over time.

After completion of the laboratory phase of the study, salmon from each group (except PARR) were gradually acclimated to seawater and transferred to a sea cage. No mortalities occurred during acclimation or transport. During 5 mo, growth rates in the SNP, L24OCT, and L24DEC groups were similar, whereas growth rate of the L24 group was half that of the other groups (Table 1). Plasma osmolarity in early December was not significantly affected by prior photoperiod treatment, nor were there significant differences in gill Na^+,K^+-ATPase activity between SNP and L24 fish. Plasma T4 and T3 were highest in the SNP group and lowest in the L24 group and were significantly different from one another. Plasma T3 was also significantly lower in both the L24OCT and L24DEC groups relative to the SNP group, in spite of their similar growth rates.

Discussion

Exposure of Atlantic salmon to continuous light from first feeding did not increase growth rate relative to those exposed to normal photoperiod. The change from continuous light to normal photoperiod (a decrease in day length) resulted in decreased growth (Fig. 2). In contrast, exposure of juvenile Atlantic salmon to continuous light starting in November (increase from 9 to 24 h day length) resulted in increased growth during the next 6 mo (Saunders et al. 1985). It appears that it is not continuous light *per se* that has a beneficial effect on growth, but rather an increase in day length.

Under conditions of SNP, Atlantic salmon undergo increases in salinity tolerance, gill Na^+,K^+-ATPase activity, and plasma T4 levels in spring (Fig. 3). Continuous light, when applied from first feeding or in autumn, inhibits increases in salinity tolerance and gill Na^+,K^+-ATPase activity (Fig. 4; Saunders et al. 1985). These results are in apparent conflict with those of Wagner (1974b) in which seawater survival and hypoosmoregulatory ability of steelhead (*Salmo gairdneri*) maintained under continuous light was similar to that of fish raised under normal photoperiods. In Wagner's experiments, fish were either (1) exposed to continuous light soon after hatching but were challenged with seawater at small sizes (<10 cm, a size too small for this species to undergo transformation) or (2) challenged at larger sizes but only after exposure to continuous light late in the year when the fish may have been developmentally prepared to perceive and respond to continuous light as an increase in day length. The size dependence of spring increases in salinity tolerance and gill Na^+,K^+-ATPase activity in salmonids has been demonstrated before (Parry 1960; Conte and Wagner 1965; Clarke et al. 1978; Boeuf et al. 1985).

Species and/or experimental differences, such as our use of 40‰ to test salinity tolerance versus the 30–32‰ used by Wagner (1974b), may also account for the dissimilar results between studies.

Other experiments indicate that conditions of constant day to night ratio do not inhibit behavioral or physiological changes associated with the parr–smolt transformation. Eriksson and Lundqvist (1982) subjected 1+-yr-old Atlantic salmon to a 12:12 day:night cycle. Body silvering and fin margin darkening, condition factor, and growth rate cycled with an endogenous rhythm of 10 mo. Our results indicate that continuous light applied early in ontogeny prevents an endogenous rhythm associated with parr–smolt transformation. Continuous light may act directly on the pineal and other photoreceptors to cause arhythmia. This inhibition is reversible, since Atlantic salmon returned to normal photoperiod underwent normal transformation, albeit with a significant lag period in the L24DEC group. Sexual maturation of presmolt male Atlantic salmon, which is also under photoperiod control in salmonids (Eriksson and Lundqvist 1980), is not inhibited by exposure to continuous light (R. L. Saunders, unpubl. data).

Photoperiod manipulation alters the timing of the parr–smolt transformation in several salmonid species, indicating that increasing day length is a zeitgeber (time-keeping device) for transformation. Decreasing or increasing the rate of change in day length retards or accelerates, respectively, increases in gill Na^+,K^+-ATPase activity and downstream migratory behavior in steelhead (Zaugg and Wagner 1973; Wagner 1974b). Sockeye (*Oncorhynchus nerka*) and coho (*O. kisutch*) salmon respond to increasing daylength with increased hypoosmoregulatory ability but show no change under constant or decreasing photoperiod (Clarke et al. 1978). Doubling the rate of increase in day length advanced the seasonal cycle of hypoosmoregulatory ability of a Baltic Sea stock of Atlantic salmon by 1 mo (Clarke et al. 1985).

Our results also support the role of increasing day length in stimulating transformation. Atlantic salmon exposed to continuous light from first feeding and returned to normal photoperiod in late October underwent increases in salinity tolerance and gill Na^+,K^+-ATPase activity the following spring, concurrent with fish under SNP. Fish returned to SNP in late December also had increases in these parameters but with a delay of 1 mo (Fig. 4). This "lag" period may have resulted from the decrease in day length experienced by these fish when returned to normal photoperiod from continuous light. Atlantic salmon larger than 14 cm which were raised under normal photoperiod and then exposed to continuous light in January (change from 9 to 24 h day length) responded with increased gill Na^+,K^+-ATPase activity (S. D. McCormick and R. L. Saunders, unpubl. data). Similarly, Komourdjian et al. (1976) found that when photoperiod was changed from 8 to 16 h day length in mid-December, salinity tolerance, apparent activity of pituitary somatotropes, growth, and smolt appearance of Atlantic salmon (>13 cm) occurred 2 mo earlier than in fish maintained under SNP.

The present experiments do not indicate whether rate of change in day length, a threshold day length, or some combination of the two is necessary to stimulate the parr–smolt transformation. Clarke et al. (1978) concluded that effects of photoperiod on growth and seawater adaptation in underyearling sockeye and coho salmon were not related to the accumulated hours of day length but to the direction and rate of change in day length. These authors also suggested that sensitivity to photoperiod changed with season or prior photoperiod experience. This hypothesis may explain the results of our current study, but we cannot exclude the possibility that a threshold day length is also involved in the parr–smolt transformation of Atlantic salmon.

Simultaneous measurements of salinity tolerance, gill Na^+,K^+-ATPase activity, and thyroid hormones during environmental manipulation of the parr–smolt transformation have not been reported previously. Our results indicate that inhibition or phase-shift of increases in salinity tolerance is accompanied by similar changes in gill Na^+,K^+-ATPase activity (Fig. 4). The strong correlation between these parameters (Fig. 5) is necessary but not sufficient to establish a causal relationship. This correlation underscores the utility of measuring gill Na^+,K^+-ATPase activity as an indicator of salinity tolerance and potential for growth in seawater. However, changes in salinity tolerance can occur rapidly, especially in warmer water (R. L. Saunders, unpubl. data), and this correlation may not hold when entrance into seawater is preceded by a lengthy migration (Ewing et al. 1985). Changes in condition factor (Fig. 2) were also affected by photoperiod treatment, decreasing concurrently with increases in salinity tolerance and gill Na^+,K^+-ATPase activity. The value of condition factor as a smolt criterion has been previously demonstrated by Farmer et al. (1978) and may reflect a complex interaction between growth and metabolism (McCormick and Saunders 1987).

Plasma T4 levels were influenced by photoperiod treatment, and some relationship between plasma T4 levels and osmoregulatory changes was apparent. When plasma T4 was at its peak in the SNP and L24OCT groups, levels in the L24DEC and L24 groups were significantly lower. However, the shifts in salinity tolerance and gill Na^+,K^+-ATPase activity in the L24DEC group were not accompanied by a shift in their plasma T4 levels. Furthermore, the inhibition of osmoregulatory changes by continuous light was not accompanied by an inhibition of the seasonal plasma T4 cycle. Plasma T3 levels were not affected by the photoperiod treatments, nor were changes in plasma T3 correlated with increases in salinity tolerance and gill Na^+,K^+-ATPase activity.

Exogenous thyroid hormones do not increase gill Na^+,K^+-ATPase activity or result in permanent increases in salinity tolerance that are independent of hormonally induced increases in body size (Miwa and Inui 1985; Saunders et al. 1985; Omeljaniuk and Eales 1986). A direct causal role of thyroid hormones in triggering the parr–smolt transformation, analogous to their role in inducing amphibian metamorphosis (see Freiden 1967), remains to be demonstrated. A more likely role for thyroid hormones is a permissive or synergistic one involving interactions with other neuroendocrine factors (see Dickhoff and Sullivan 1987). In support of this hypothesis, Miwa and Inui (1985) found that T4 and growth hormone administered together increased salinity tolerance and gill Na^+, K^+-ATPase activity of Amago Salmon to a greater extent than growth hormone alone. Similar evidence for a permissive or synergistic role for thyroid hormones in control of growth and development has been reviewed by Donaldson et al. (1979).

In addition to the dramatic seasonal increase in salinity tolerance of smolts, seasonal changes in seawater adaptability may occur throughout salmonid ontogeny (Hoar 1965, 1976; Wagner 1974b; Saunders and Henderson 1978; Langdon and Thorpe 1985; McCormick and Saunders 1987). In the present study, the PARR group exhibited a 40% increase in gill Na^+,K^+-ATPase activity in April which declined steadily thereafter. Although this increase was small relative to that observed in larger fish, it suggests that a seasonal increase in gill Na^+,K^+-ATPase activity does occur in parr. No change in salinity tolerance was detected in the PARR group during this period, perhaps due to the high salinity used in the present study. Spring increases in salinity tolerance have been observed in small (10–12 cm) presmolt steelhead (Conte and Wagner 1965). Plasma T4 concentrations of the PARR group in late spring rose to levels that were two-thirds that of the SNP group 6 wk earlier (Fig. 6). A similar spring increase in circulating thyroid hormones has also been observed in presmolt coho salmon and was hypothesized to be an important "maturational" event influencing transformation the following spring (Dickhoff et al. 1982; Dickhoff and Sullivan 1987).

Inhibition of increases in gill Na^+,K^+-ATPase activity and salinity tolerance by exposure to continuous light did not preclude gradual adaptation of the L24 group to 30‰ seawater. Although survival and capacity to maintain plasma osmolarity after 5 mo in seawater was not significantly different from the other groups, the growth of the fish during this period was halved. This reduction in growth is due to salinity, since we have recently found that poor growth does not result if L24 fish are maintained in freshwater (S. D. McCormick and R. L. Saunders, unpubl. data). It seems likely that endogenous and environmental factors which affect changes in osmoregulatory physiology during the parr–smolt transformation will also affect growth in seawater.

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Population and Production Dynamics of an Enchytraeid Worm in a Subtropical Blackwater River

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Stites, D. L. 1987. Population and production dynamics of an enchytraeid worm in a subtropical blackwater river. Can. J. Fish. Aquat. Sci. 44: **1469–1474.**

Population and production dynamics of *Barbidrilus paucisetus* Loden and Locy (Oligochaeta: Enchytraeidae) in a sixth-order blackwater river are described. This worm was the dominant member of the meiofauna of the sand habitat, accounting for 50–90% of the total density. Population density varied seasonally, increasing from < 20 000/m^2 in the winter to 60–120 000/m^2 in the summer. Biomass averaged 145 mg dry mass/m^2 with a range of 8–326 mg/m^2. Density peaked in the clean coarse sand of the center channel areas and was lower where the sand was finer and where more organic matter was present. Size frequency histograms indicated that there were three recruitment peaks during the sampling year, with mature and new individuals present in all seasons. Annual production (2.12 g dry mass/m^2, SE = 0.15 g/m^2), was estimated using the size frequency method and a cohort production interval correction of 3. Annual production was low compared with that of terrestrial Enchytraeidae and comparable with that of many aquatic insect species. Annual production/biomass ratio (14.3) was higher than terrestrial Enchytraeidae, due to the comparatively short generation time of this species.

On décrit la démécologie et la dynamique de production de *Barbidrilus paucisetus* Loden et Locy (Oligochaeta : Enchytraeidae) dans une rivière du sixième ordre. Ce ver dominait la méiofaune des sables où il représentait de 50 à 90 % de la densité totale. La densité des populations variait selon la saison : elle est passée de < 20 000/m^2 en hiver à 60–120 000/m^2 à l'été. La biomasse, dont la gamme allait de 8 à 326 mg/m^2, s'élevait en moyenne à 145 mg masse sèche/m^2. La densité atteignait une pointe dans le gros sable propre des zones centrales du chenal et était plus faible là où le sable était plus fin et la matière organique plus abondante. Des histogrammes de la fréquence des tailles ont révélé la présence de trois pointes du recrutement pendant l'année d'échantillonnage; des individus matures et de nouvelles recrues étaient présents à toutes les saisons. La production annuelle (2,12 g masse sèche/m^2, ET = 0,15 g/m^2) a été déterminée à l'aide de la méthode de la fréquence des tailles et d'un intervalle de production de la cohorte de 3. La production annuelle était faible par rapport à celle des Enchytraeidae terrestres et semblable à celle de nombreuses autres espèces d'insectes aquatiques. Le rapport production/biomasse (14,3) annuel était plus élevé que celui observé chez des Enchytraeidae terrestres à cause du court temps de génération de l'espèce.

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The analysis of benthic invertebrate production in streams has until very recently focused on organisms and communities inhabiting relatively coarse substrata, such as cobble and rock, in medium and high gradients. In lowland river systems, coarse substrata, including wood (snags), provide only a small fraction of the total available habitat. Coarse shifting sands often account for most of the space available in these environments. Traditionally considered an unfavorable habitat for macroinvertebrates (Hynes 1970), several studies have described a diverse micro and meiofaunal psammon community, with macroinvertebrates dominated by high densities of a few small, highly specialized insect species (Barton and Lock 1979; Benke et al. 1984; Berner 1951; Niewestnova-Shadina 1935; Soluk 1985; Whitman and Clark 1984). Secondary production in the sand may be quite important to measures of total energy flow through stream systems (Benke et al. 1984; Soluk 1985).

Oligochaetes are a major component of freshwater communities in a variety of habitats (including sand), yet unlike the arthropods with which they coexist, their production has rarely been measured. The general paucity of information on oligochaete production dynamics is due at least in part to often complex life cycles, indeterminate growth patterns, and the taxonomic intractability of immature individuals. Most of the production estimates that have been made involve the Tubificidae, probably because of their large size, ubiquity, and abundance, as well as their potential role in the food web and bioturbation of sediments (Brinkhurst 1980). The methods used vary, but generally do not include cohort or age-class dependent approaches which are usually difficult to apply to oligochaete populations. Additionally, production has most often been measured in lentic habitats. With few exceptions (Bird 1982; Lazim and Learner 1986), lotic oligochaete production has been estimated as the product of mean biomass estimates and literature-derived production/biomass (*P/B*) ratios (e.g. Berrie 1972; Benke et al. 1984; Smock et al. 1985).

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Barbidrilus paucisetus Loden and Locy (Oligochaeta: Enchytraeidae) is a freshwater species found in large rivers and freshwater bays in the eastern United States (Loden and Locy 1980; Seagle and Wetzel 1982). The Enchytraeidae are an hermaphroditic, predominantly terrestrial family, but a few species are totally adapted to freshwater habitats and may be numerically significant at particular sites (Giani and Lavandier 1977; Healy and Bolger 1984). This paper analyzes the life history, population dynamics, and production of *B. paucisetus* in a Georgia stream. The development of distinct cohorts and the relatively simple life cycle of this easily identified species allows accurate description of several aspects of its ecology.

Materials and Methods

The study site was located on the Ogeechee River, a sixth-order blackwater river in the Lower Coastal Plain of Georgia, 63 km from the river mouth (lat. 32°08′, long. 81°21′). Mean annual discharge from 1936 to 1981 was $66.8\ m^3/s$ with a range of $3.7-850\ m^3/s$ (United States Geological Survey 1982). Daily water temperatures ranged from 3.5 to 32°C with an annual mean in 1982 of 18°C. The lower Ogeechee is a meandering river with a low (0.02%) slope and broad (1 km) floodplain. The main channel is characterized by many sharp bends and short straight stretches. The bottom is 80–90% clean, loose sand. Mud and organic matter overlays the sand in backwaters (sloughs) and depositional areas of the main channel and comprises the remainder of the river bottom. *Barbidrilus paucisetus* was only occasionally present in low densities at these muddy sites ($< 500/m^2$). I report here the results of sampling the "clean" sand habitat.

Two sections of the main channel were selected as representative of the range of conditions present in the sand habitat. The first was a straight narrow section approximately 20 m wide and long, with the thalweg near center channel. The second was a 35-m-wide, 20-m-long section at the center of a river bend approximately 100 m downstream of the first section.

Each section was divided into thirds (left, center, and right strata) and three samples collected from each stratum in each section. The boat from which the samples were collected was anchored within each stratum at random by drifting in from upstream and anchoring at the end of an anchor line (approximately 10 m long) cast during the drift. The samples were collected as the boat moved freely at the end of the rope (an arc of 1–3 m) and were taken from either side and the front of the boat, providing wide spatial dispersion of the samples within a stratum.

Sampling was conducted at approximately 4-wk intervals from 15 December 1981 to 3 December 1982 (13 sample dates, 18 samples per date). I sampled with a corer on a pole (sample area = $23.76\ cm^2$) which was especially designed for use in the loose sand substrata encountered in this study (Gillespie et al. 1985). Cores averaged 25 cm in depth.

Within 8 h of collection, each sample was placed in a bucket and all animals were flushed from the sand by elutriation onto a 100-μm Nitex sieve. The sample was then fixed in Kahle's Fluid (Pennak 1978) or 70% EtOH and brought to the laboratory.

The sediment of two cores from each stratum on each section was saved after elutriation in February, March, June, August, October, and December. Each sample was dried, sieved on a mechanical sieve shaker, and analyzed following the procedures described in Folk (1974).

Barbidrilus paucisetus were counted and measured at 12–50× with a dissecting microscope. The species is quite distinctive and easily distinguished from other organisms. Since these animals are fragile and easily fragmented, the number of prostomia present was taken as the total number of individuals. If the total count was less than 100, all whole individuals were measured and placed in millimetre size classes (0.5, 1.5, 2.5, 3.5, 4.5, 5.5, 6.5, and 7.5 mm). The size class distribution of organisms was then adjusted to reflect the total count. If more than 100 individuals were present the first 100 whole individuals encountered were measured and the total sample assumed to follow that size class distribution.

The average volume of individuals at the midpoint of each length class was estimated from measurements of the length and mid-body width of over 250 individuals spanning all length classes. As the body width of an individual changes little except at the two ends, we assumed a right cylinder relationship between width, length, and volume. A volume to dry mass ratio was determined by calculating (as above) the volumes of five sets of freshly collected individuals ($n = 5-10$) ranging from 1.0 to 6.0 mm in length and then lyophilizing each set of animals to dryness on preweighed aluminum boats. The dry mass of each set was measured to the nearest 0.1 μg on a Cahn 29 Automatic Electrobalance and the average ratio of dry mass to total volume for the samples calculated. Average individual volume in each length class was multiplied by this fraction to obtain average individual dry biomass in each length class.

Density and biomass statistics were calculated using the SAS programs (SAS Institute Inc. 1982). Variance estimates were calculated as described in Elliott (1977) for stratified random samples. Production was calculated using a production program written by D. M. Gillespie and modified by this author. The program provided size frequency production and production variance estimates by size class as described in Krueger and Martin (1980).

Results

Morisita's index of dispersion I_δ (Morisita 1959; Elliott 1977) calculated for the samples from each collection date showed the population to be contagiously distributed throughout the year. Index values were all significantly different ($p < 0.01$) from a random distribution. Maximum contagion occurred in the December 1981 ($I_\delta = 4.6$) and January 1982 ($I_\delta = 9.9$) collections and in the collections taken between September and December ($3 < I_\delta < 5$). Most other I_δ values ranged between 2 and 3. As a consequence, standard errors for the population density and biomass estimates were derived from data transformed according to Taylor's power law (Taylor 1961) following the methods of Elliott (1977). Power law regressions for numerical abundance and biomass data were highly significant ($p < 0.0001$):

$$\text{Abundance: } \log s^2 = 0.261 + 1.939 \log (x)\ r^2 = 0.96$$

$$\text{Biomass: } \log s^2 = 0.249 + 1.719 \log (x)\ r^2 = 0.85$$

where x = replicate mean and s^2 = variance of the mean.

Dry mass averaged 20.2% of volume. This value is very similar to volume – dry mass and wet – dry mass relationships described for Enchytraeidae, other oligochaetes, and other invertebrates (Driver 1981; Lakhani and Satchell 1970; Neves 1979; Standen 1973; Waters 1977).

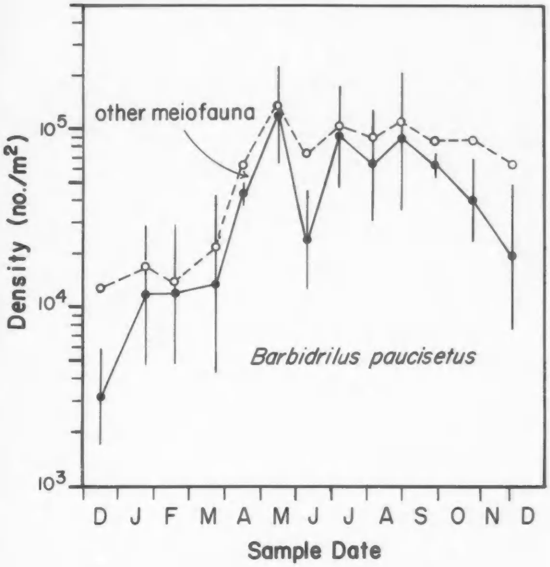

FIG. 1. Cumulative mean density of *Barbidrilus paucisetus* and other meiofauna by sampling date. Bars are 1 standard error on either side of the mean value for *B. paucisetus* on each sample date.

TABLE 1. Annual average density (no./m^2) in each section and stratum.

Section	Average	Left	Center	Right
1	79 774	35 129	101 859	101 719
2	12 976	4 360	14 591	20 074

Average monthly population densities of *B. paucisetus* ranged from 3157 to 122 000/m^2 (yearly average = 47 408/m^2) and showed a strongly seasonal pattern (Fig. 1). Density increased in the spring from the winter low levels and maintained densities of 60 000 – 120 000/m^2 until late in the fall. The population resided predominantly in section 1 where average annual densities of 100 000/m^2 were found in the center and right strata (Table 1). Densities in section 2 were much lower and declined from the inside to the outside of the river bend.

Barbidrilus paucisetus usually made up more than two thirds of the total meiofauna and only on three occasions accounted for less than 50% of the total (Fig. 1). The density of all other meiofauna combined averaged 20 720/m^2. Dominant taxa included Turbellaria (6025/m^2), Nematoda (5923/m^2), Bathynellacea (3858/m^2), and Ostracoda (3482/m^2). Also present were lesser densities of Copepoda, Hydracarina, and Amphipoda. Rotatoria and Tartigrada were only rarely found, although described by Pennak (1978) as common to abundant in a variety of sand substrata. The total density of the macrofauna (other oligochaetes, insects, and molluscs collected in samples) averaged 11 428/m^2 over the sample period (Stites 1986).

Biomass (Fig. 2) followed the seasonal density pattern, ranging from 8 to 326 mg dry mass/m^2 with a yearly average of 145 mg/m^2. The reason for the low June values (Fig. 1, 2) is not apparent. Size frequency analysis (below) suggested no sudden change in the population.

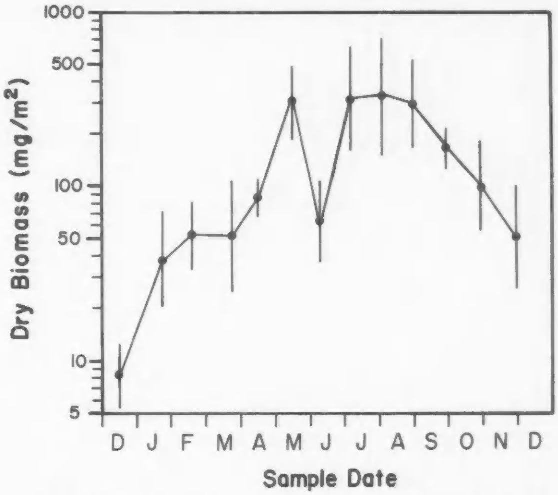

FIG. 2. Mean biomass of *Barbidrilus paucisetus* by sampling date. Bars are 1 standard error on either side of the mean value.

TABLE 2. Quartile analysis of grain size composition of the six strata. Q_1, Md, and Q_3 are grain sizes (mm) of 25, 50, and 75% percentiles of average cumulative sample sediment mass. Spread and skew are in phi units.

Stratum	Q_1	Md	Q_3	Spread	Skew
Section 1					
Left	0.81	1.22	1.89	1.22	0.26
Center	1.11	1.56	2.22	1.00	0.00
Right	1.27	1.74	2.46	0.95	−0.05
Section 2					
Left	0.55	0.97	1.41	1.34	−0.12
Center	0.76	1.36	2.46	1.70	0.00
Right	1.00	1.50	2.30	1.20	0.00

Sediment grain size analysis suggested that *B. paucisetus* was most successful in strata containing very uniform coarse sand. Median and mean grain sizes were largest at strata of higher densities in both sections (Table 2). The range of grain sizes (as quartile spread) was generally higher in the river bend (section 2) strata but relatively evenly distributed around the median value of grain size of each stratum. Very fine sediments and organic matter collected in the samples were not analyzed, as they were washed out during the elutriation process. However, J. L. Meyer (Department of Zoology, University of Georgia, Athens, GA 30602, USA, pers. comm.) has found significantly more organic matter in the left stratum on section 1 than in the other strata in that section. Qualitatively the strata with lower densities contained greater amounts of silt and organic matter, and the outside of the river bend (the left stratum of section 2) appeared to contain more fine sediments than any other site.

The life history pattern of the population was analyzed by percent size frequency histograms of each sample set (n = 18). Three cohorts developed over the sample period (Fig. 3). A winter generation developed between December 1981 and February–March 1982. A second cohort was recruited in April. The spring cohort matured and produced a

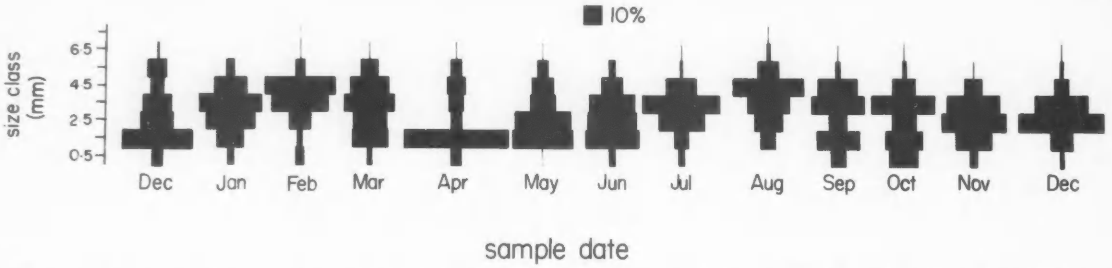

FIG. 3. Size frequency distribution by sampling date. Width of each bar represents the percentage of the total collection found in a given size class.

TABLE 3. Size frequency production estimate for *Barbidrilus paucisetus* in the Ogeechee River, Georgia, 15 December 1981 – 3 December 1982. Data are based on 18 samples per date on 13 sampling dates at approximate 4-wk intervals. Biomass and production values are for dry mass. Average biomass = 0.1484 g, uncorrected production = 0.7072 g, annual production (SE) = 365/122 × 0.7072 = 2.122 (0.149) $g \cdot m^{-2} \cdot yr^{-1}$.

Size class (mm)	Mean individual mass (mg)	No./m^2	Biomass (g)	Number lost	Mass at loss (mg)	Size class production (g)
0.5	0.00012	2 135	0.00025	−8868	0.00026	(−0.01872)
1.5	0.00059	11 004	0.00649	350	0.00105	0.00294
2.5	0.00187	10 653	0.01989	−2216	0.00258	−0.04567
3.5	0.00355	12 869	0.04574	4425	0.00470	0.16637
4.5	0.00622	8 445	0.05248	6548	0.00773	0.40504
5.5	0.00962	1 897	0.01825	1499	0.01120	0.13439
6.5	0.01305	398	0.00519	390	0.01385	0.04322
7.5	0.01469	7	0.00011	7	0.01469	0.00087

TABLE 4. Size frequency production (g dry mass$\cdot m^{-2} \cdot yr^{-1}$) and standard error (SE) estimated by stratum and section. Each value estimated from three replicate samples per sampling date. CPI = 122.

Section	Left (SE)	Stratum center (SE)	Right (SE)	Section average (SE)
1	1.711 (0.156)	4.541 (0.362)	5.252 (0.557)	3.655 (0.259)
2	0.272 (0.083)	0.573 (0.060)	1.240 (0.231)	0.558 (0.088)

third less evident cohort in late summer. This third cohort had reached the 2.5-mm size class by 3 December 1982.

Production was estimated by the size frequency method with the cohort production interval (CPI) correction (Hynes and Coleman 1968; Hamilton 1969; Benke 1979) for all data, each section, and each stratum. Production for all samples was 2.12 g dry mass$\cdot m^2 \cdot yr^{-1}$ (SE = 0.149 g/m^2) (Table 3). Size class densities were not adjusted according to time spent in each size class, as suggested by Hamilton (1969), as this could not be estimated from field data. Thus, smaller size classes were underrepresented in the analysis. Benke and Wallace (1980) suggested that the apparent negative production that results from this (i.e. the first value in the last column of Table 3) could be ignored in the production summation. All such negative values at the top of the column and their associated variance estimates were eliminated from the estimates. All other negative values were included. The difference in the production variance was usually less than the least significant digit reported. The cohort $P/B = 4.8$ is within the expected range (Waters 1977) and yields an annual $P/B = 14.3$.

A more conservative analysis of Fig. 3 would suggest 2.5 generations a year or a CPI (average development time) of 146 d. This would result in an annual production estimate of 1.77 g dry mass$\cdot m^2 \cdot yr^{-1}$ and an annual $P/B = 11.9$. Several factors led me to use the shorter CPI value in the production estimate. All individuals in the 4.5-mm and larger size classes were mature (personal observation). Due to the presence of these size classes on all sampling dates (Fig. 3) the population was potentially capable of some reproduction all year long. In addition, individuals in the smallest size class were present in all except one collection, suggesting some recruitment all year. Recruitment was probably high for long periods around each recruitment peak. Using a higher CPI value would likely have underestimated the actual production.

Production was also calculated separately for each section, and each stratum in each section (Table 4). Elimination of consecutive negative production values in the smallest size classes generally resulted in only minor (< 10%) increases in the annual production estimates. All but a small fraction of the total production occurred in section 1. Production in the highest density strata of section 1 was twice that of the production averaged over all samples and 20 times that for the lowest density stratum of section 2. Enchytraeid production in section 1 strata accounted for 10% (left stratum), 36% (center stratum), and 27% (right stratum) of total invertebrate (oligochaete, insect, and mollusc) production there (Stites 1986). Only 1–3% of the total production in section 2 strata was accounted for by the enchytraeid (Stites 1986).

Discussion

Published psammon community biomass estimates (mostly Insecta) range from < 100 to 500 mg dry mass/m^2 with average densities as high as 77 000/m^2 (table 3 in Soluk 1985). The available production estimates for psammophilous species and species groups generally range between 0.5 and 2.0 g dry mass$\cdot$m$^{-2}\cdot$yr^{-1} (Benke et al. 1984; Smock et al. 1985; Soluk 1985). The production of *B. paucisetus* (2.12 g dry mass$\cdot$m$^{-2}\cdot$yr^{-1}) accounted for almost 10% of total sediment production measured in 1982 (Stites 1986) and indicates the importance of non-insect fauna to the overall production and energy flow of lowland rivers. *Barbidrilus paucisetus* production estimates from some strata are higher than those measured for many macroinvertebrate species (Waters 1977). Stratum-specific information also demonstrates the importance of sampling a range of microhabitats when estimating production for an entire system. Accurate description and estimation of the relative importance of each microhabitat further improves production estimates.

Production estimates for terrestrial Enchytraeidae of 11–65 g live mass$\cdot$m$^{-2}\cdot$yr^{-1} (approximately 2–12 g dry mass$\cdot$m$^{-2}\cdot$yr^{-1}), and information on life histories, growth rates, and longevity, indicate that annual P/B ratios in the neighborhood of 2.5 are likely for most species (Standen 1980). The estimated annual P/B of 14.3 for *B. paucisetus* is the result of a shorter life cycle than those of terrestrial Enchytraeidae.

Studies of oligochaete growth or life history in warm lotic systems are rare. Life history studies (predominantly from temperate lentic habitats) have described the development of four to five or fewer cohorts per year for a variety of sexually reproducing Tubificidae (Andreani et al. 1984; Johnson and Brinkhurst 1971; Kennedy 1966; Ladle and Bird 1984; Poddubnaya 1980). Aston (1968) found that *Brachiura sowerbyi* completed its life cycle in 4–12 mo in a temperate river (the Avon, annual average temperature 10°C) and in the warmer (average 15°C) temperatures of a power station effluent discharging into the Thames River. Growth of immature worms and cocoon production was maximal at near 25°C, however.

Most production estimates for sexually reproducing oligochaetes in running waters are based on standing stock biomass estimates combined with literature-derived CPI and turnover rates (e.g. Benke et al. 1984; Berrie 1972; Smock et al. 1985). Lazim and Learner (1986), however, estimated production of *Tubifex tubifex* by the instantaneous growth method. Production of 139 g dry mass$\cdot$m$^{-2}\cdot$yr^{-1}, with an annual $P/B = 3$, was estimated for the organically enriched, temperate (average temperature 11°C) stream they studied. Published estimates of annual P/B ratios reach as high as 12 (Johnson and Brinkhurst 1971; Potter and Learner 1974), but more generally, productivity is estimated to be less than this (Waters 1977). Johnson and Brinkhurst (1971) offered evidence that lentic macrobenthic community turnover ratios could be approximated as $T^2/10$ where T is mean annual temperature. This suggests an expected annual turnover in the Ogeechee River of approximately 32 (mean temperature 18°C). On this basis, *B. paucisetus* has a lower turnover rate than might be expected for temperate conditions.

There are some similarities in the results of lotic insect population studies and this present research. Soluk (1985) studied the population dynamics and production of small psammophilous Chironomidae (Insecta: Diptera) in a lowland river. He described high-density populations with unexpectedly long (> 3 mo) development times even during warm water (mean temperature 19.5°C) periods. Stites (1986) measured comparatively low growth rates for psammophilous chironomids in the Ogeechee River. In contrast, most lotic chironomids studied display rapid growth and short development times (days or weeks) at high temperatures (Mackey 1977; and see Benke et al. 1984). Soluk suggested that food limitation or disturbance stress might explain his findings. Food limitation seems unlikely to be an important influence on the growth of psammophilous species in the Ogeechee River, as the amount of organic matter stored in the sediments of the Ogeechee River is quite high (2.7 kg AFDM/m^2) and largely less than 1 mm in size (Benke and Meyer 1987). Slow individual growth in high-density populations may be a common adaptation to life in loose sand.

There is some evidence that, based on size, meiofaunal populations generally exhibit lower productivity than is common for macrofaunal populations (Banse and Mosher 1980; McIntyre 1969). Scaled by mass at maturity (0.0062 mg, 4.5 mm size class, Table 3), *B. paucisetus* has a lower annual P/B (14.3) than expected for most benthic invertebrates, similar to those of free-living nematodes and other (mostly marine) meiofaunal populations (fig. 6 in Banse and Mosher 1980; for *B. paucisetus*, log mass in kilocalorie equivalents and log P/B are −4.51 and 1.15, respectively). An accurate assessment of hypotheses concerning the productivity of meiofauna in different habitats awaits further studies of production dynamics, particularly in freshwaters.

This study is among the first to provide direct estimates of the contribution of non-arthropod benthic meiofauna to riverine production. It is clear that this species is an important contributor to biotic energy flow through the sand habitat of this river. Direct estimation of the production of other abundant meiofauna such as Turbellaria and Nematoda will probably increase the significance of this generally overlooked component of the lotic benthos.

Acknowledgements

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Responses of Juvenile Atlantic Salmon (*Salmo salar*) to Episodic Increases in Acidity of Nova Scotia Rivers

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Lacroix, G. L., and D. R. Townsend. 1987. Responses of juvenile Atlantic salmon (*Salmo salar*) to episodic increases in acidity of Nova Scotia rivers. Can. J. Fish. Aquat. Sci. 44: 1475–1484.

Toxicity of different pH levels to Atlantic salmon (*Salmo salar*) parr held in floating pens for 54 d was assessed in four acidic streams of Atlantic Canada during the autumnal episode of increasing acidity. All parr died in the two streams where pH levels decreased below pH 4.7. Mortality was related to the rate of pH change and to parr size. Plasma sodium and chloride concentrations decreased by about 50–70 $mmol \cdot L^{-1}$ in parr of these two streams, and the rate of electrolyte loss was related to the rate and severity of pH change. In the other two streams, pH levels were never lower than pH 4.8 and no fish died. Plasma sodium and chloride concentrations in these parr decreased by 20–30 $mmol \cdot L^{-1}$, but only after 30 d of exposure. Increased hematocrit, increased respiratory–cough response, and decreased feeding response, especially for parr in the most acidic streams, were also the result of stress apparently related to decreasing pH levels. Concentrations of labile aluminum were usually less than 10% of total dissolved aluminum and always less than 50 $\mu g \cdot L^{-1}$ in the four streams. Accumulation of aluminum in the gills of parr was not a significant factor in the lethal effects observed, which were more likely due to low pH.

La toxicité de différents taux de pH sur les tacons du saumon atlantique (*Salmo salar*) tenus dans des cages flottantes pour une durée de 54 jours a été évaluée dans quatre rivières acides de la région atlantique du Canada lors de l'épisode automnale d'accroissement de l'acidité. Tous les tacons moururent dans deux des rivières où il y eu une baisse des taux de pH sous 4,7. La mortalité était en fonction de la vitesse de changement du pH ainsi qu'à la taille des tacons. Les concentrations de sodium et de chlorure plasmatiques des tacons de ces deux rivières diminuèrent d'au moins 50 à 70 $mmol \cdot L^{-1}$. La vitesse de perte de ces électrolytes variait en fonction de la vitesse et de la sévérité des changements du pH. Les taux de pH des deux autres rivières ne furent jamais moindre que pH 4,8 et aucuns poissons ne sont morts. Les concentrations de sodium et de chlorure plasmatiques de ces tacons diminuèrent par 20 à 30 $mmol \cdot L^{-1}$, ceci seulement après 30 jours d'exposition. Un hématocrite élevé, une fréquence de respiration et de toux élevée, et une perte d'apétit chez les tacons des rivières les plus acides se sont aussi avérées comme résultats du stress relié au décroit des taux de pH. Les concentrations d'aluminium sous forme labile étaient habituellement moindre que 10% de l'aluminium dissous total et étaient toujours en dessous de 50 $\mu g \cdot L^{-1}$ dans les quatres rivières. L'accumulation de l'aluminium dans les branchies des tacons ne fut pas un facteur d'importance en ce qui a trait aux effets létaux que l'on a observé, lesquels étaient plutôt dus aux bas taux de pH.

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The acidification of surface waters, and its effects on river chemistry, can subject fish to a wide range of toxicological effects. These may be both direct effects of acidity and indirect effects due to changes in the concentration and forms of toxic metals. The decline and loss of Atlantic salmon (*Salmo salar*) populations and other salmonids from acidified rivers in Scandinavia have been attributed to the combined effects of H^+ and metals such as Al and Fe (Muniz 1981; Andersson and Nyberg 1984; Fivelstad and Leivestad 1984; Rosseland and Skogheim 1984; Skogheim and Rosseland 1984; Skogheim et al. 1984).

In Canada, major losses of Atlantic salmon populations have occurred in acidic rivers of Nova Scotia (Watt et al. 1983), but identification of the salmon life cycle stages affected and of the factors involved is incomplete. In these rivers, significant mortalities of Atlantic salmon eggs and fry occur when pH levels decrease to pH 5.0 or less (Lacroix 1985a; Lacroix et al. 1985). Sublethal physiological effects have also been measured in salmon parr and smolts at pH levels of about 5.0–5.5 (Lacroix 1985b). These lethal and sublethal effects have been attributed to high H^+ concentrations at low environmental Ca concentrations, but the potential toxicity of metals such as Al and Fe has not been directly resolved.

The Nova Scotia waters of concern have high Al and Fe concentrations, but unlike the Scandinavian rivers they are highly colored and have high concentrations of fulvic and humic acids (Farmer et al. 1980; Oliver et al. 1983). The acidity of these waters is thought to be affected both by organic acids from peatland and bogs in the catchments and by acid deposition from long-range sources (Gorham et al. 1986; Kerekes et al. 1986; Underwood et al. 1987). Minimum pH

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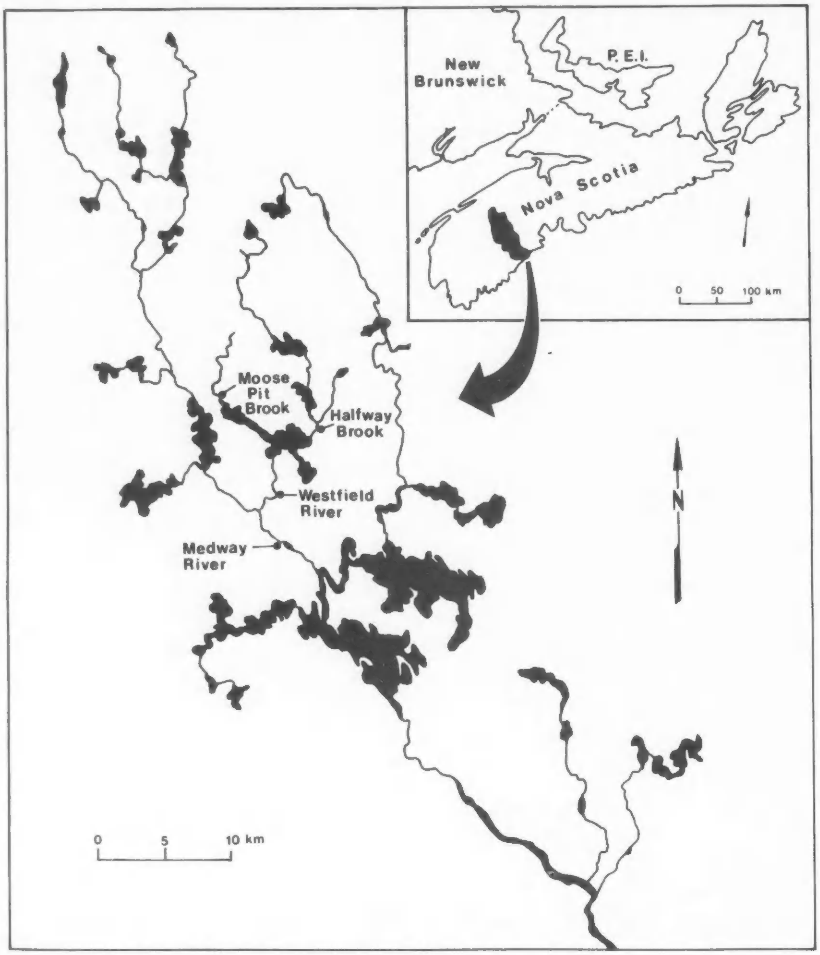

FIG. 1. Rivers and sites in Nova Scotia where pens holding Atlantic salmon parr were located during autumn 1985.

levels in the salmon rivers usually occur after autumn rains and increases in water flow (Lacroix and Kan 1986). Density estimates of juvenile salmon in these acidic rivers indicate that the rate of parr loss increases at that time, possibly due to mortality (G. L. Lacroix, unpubl. data).

This study used an in situ approach to assess the toxicity, to Atlantic salmon parr, of water in four acidic rivers of different pH in a watershed of southwest Nova Scotia during the autumnal decrease in pH. The pH of these rivers differs on the basis of their position within the drainage basin; the small headwater streams are more acidic than the large rivers downstream because headwater catchments contain a larger proportion of bogs and are more readily affected by acid deposition. Physiological and behavioral reponses of the parr and the extent of Al and Fe accumulation in their gills were investigated. The responses are examined relative to chemical differences among rivers and episodic changes within each river to identify lethal and accessory factors involved.

Materials and Methods

Juvenile Atlantic salmon (1985 year-class) were obtained on October 29, 1985, from the Mersey Hatchery (Nova Scotia) and transported to sites on four rivers which had different pH levels. One hundred and twenty parr, distributed equally among two size categories (<10 and ≥ 10 cm; modes 8 and 11 cm) were randomly placed in a floating pen in each of the Medway River, Westfield River, Halfway Brook, and Moose Pit Brook (Fig. 1). Mean size of parr was similar among streams (ANOVA, $P > 0.05$; mean length 9.6 cm and mean weight 10.4 g). Each pen consisted of a 170 × 110 cm floating platform with lid and a hanging (weighted) 120 × 60 × 60 (deep) cm bag of netting (6-mm mesh size) with a 120 × 35 cm Plexiglas window along one side. The pens were anchored in small pools (depth 0.75–1.5 m) and adjusted to produce similar conditions in all pens (water velocity <10 $cm\cdot s^{-1}$). These velocity conditions are similar to those normally used in autumn by salmon parr in the wild (Rimmer et al. 1984). Parr were held in the pens until 100% mortality or until December 22 (54 d). The pens were checked daily and dead fish were removed, measured, and weighed. The parr were fed a supplemental ration of dry pellets (2% body weight) once daily except within 48 h of blood sampling.

Water samples were taken daily at each site for measurements of pH (low-conductivity combination glass electrode) and conductivity (temperature-compensated at 25°C). Water temperature (maximum−minimum) was recorded daily and dissolved oxygen concentration was regularly measured both inside and outside the pens. Water was also collected weekly, kept at 4°C, and analyzed within 10 d using standard methods (APHA et al. 1980). The pH was verified and Gran alkalinity (total inflection point) was determined by electrometric titration. Cations (Na^+, K^+, Ca^{2+}, Mg^{2+}) were measured by atomic absorption spectroscopy (AA) and anions (Cl^-, SO_4^{2-}) by ion chromatography (IC). Filtered (0.45-μm membrane porosity) and acidified (concentrated HCl, $pH < 2$) samples were analyzed for dissolved organic carbon (DOC) by the combustion−infrared method.

Additional water samples were taken, filtered (0.45-μm membrane porosity), and analyzed within 48 h for different Al fractions by graphite furnace atomic absorption (GFAA) (analytical matrix, 0.2% HNO_3, vol/vol). Total dissolved Al was measured in the filtrate and a second portion of the filtrate was treated with fractionally loaded Chelex-100 ion-exchange resin (30 min contact time) to separate potentially labile (exchangeable, inorganic) forms from nonlabile (nonexchangeable, mostly organic) forms of dissolved Al (Lacroix and Kan 1986).

Observers using diving suits and snorkeling gear quantified parr behavior. Fish were viewed from the side through the Plexiglas for improved visibility and to reduce the fright and stress often caused by observing fish from above. Three times per week, except when stream or weather conditions did not permit, 10 fish per pen were individually observed. The frequencies of the following stereotyped behaviors shown by parr (Keenleyside and Yamamoto 1962) were scored during 120 s of observation for each fish: coughing, yawning, fighting and threat behaviors (i.e. charging, frontal and lateral displays, chasing, and nipping), fleeing, chafing, and feeding (i.e. on the drift passing through the pens). The breathing rate of parr was estimated from the frequency of mouth and opercular movements scored over 60 s or, in some cases when difficulties in monitoring arose, on a series of 10-s intervals. After completion of these observations, food was added to the pens in small, approximately equal lots at intervals of 30 s and further observations of feeding behavior were made. The percentage of food pellets intercepted by all fish in each pen was estimated and the overall feeding intensity was scored on a qualitative scale as follows: no feeding (0), sluggish feeding (1), moderate (steady) feeding (2), and vigorous feeding (3). Any adjustment and cleaning of pens or removal of fish was always done after completion of observations. The schedules for observations were randomized to yield similar numbers of visits at different times of the day at each site to minimize the effects of diurnal behavior changes.

Blood was sampled from parr at the hatchery (controls) on October 29 (day 0) and after 7, 28, and 54 d. Control parr at the hatchery were also of two size categories and were held in separate tanks (1.8 m in diameter) from the regular hatchery stock. Water inflow to the tanks was filtered through limestone chips, as described by Farmer et al. (1980), to maintain pH at about 5.5 regardless of natural fluctuations in the Mersey River. Parr in the pen in each river were sampled after 3, 7, 14, 28, 42, and 54 d of exposure to natural conditions. Samples were collected from at least eight parr (four of each size category) from each site on each occasion. Sometimes, for samples of small parr, plasma from two or three parr had to be combined to provide a sufficient volume for analysis. During periods of fish mortality, seemingly normal parr and moribund parr were also sampled. Fish were individually netted, stunned with a light blow to the head, and rapidly measured and weighed (nearest 0.1 cm fork length and 0.1 g). Care was taken to minimize handling and disturbances in the pens and to quickly process fish one at a time. The caudal peduncle was blotted dry and severed, and the area of the cut was blotted. At least two heparinized microhematocrit glass capillary tubes were filled with blood flowing from the caudal artery, sealed, and immediately centrifuged for 5 min at 6300 × *g*. Hematocrit was determined and a plastic transfer pipet was slipped over the open end of the capillary tube. The tube was etched with a file and severed at the interface between plasma and packed cells. The plasma was then expelled into a polypropylene container, capped, and placed in a cooler over ice. The plasma samples were refrigerated (4°C) and analyzed within 48 h of collection.

The plasma samples were analyzed for cations (Na^+, K^+, Ca^{2+}, Mg^{2+}) by AA and Cl^- was measured by a colorimetric method (modification of method 407D, APHA et al. 1980). Plasma was gauged by weight because the viscosity of plasma often precludes a precise volumetric sample delivery. A comparative analysis of some samples was also made by IC (cations) and by coulometric−amperometric titration (Cl^-). The average relative difference between methods was less than 5% in all cases.

The gills of sampled fish were dissected out and frozen (−20°C) in separate Whirl-Pak bags. Some of these samples were later prepared and analyzed for Al and Fe content. The complete gills (all arches from both sides) were used because of a possible natural bias associated with the allometry of gill arches. The frozen gills were lyophilized at < -50°C and a pressure <30 Pa for at least 48 h and were then weighed (nearest 0.1 mg). They were incinerated in an electric muffle furnace at 450°C in Pyrex beakers for 16 h and the ashes were dissolved in 1.0 mL of concentrated Aristar HNO_3 (BDH Chemicals Ltd.) and diluted to 2% HNO_3 (final volume 50 mL) with deionized, distilled water. These samples were analyzed for Al by GFAA and for Fe by flame AA. Duplicate analytical (reagent) blanks, processed with each batch of 20 samples, were always below the detection limits ($Al < 1\ \mu g \cdot L^{-1}$, $Fe < 20\ \mu g \cdot L^{-1}$). Replicates (four) of the National Bureau of Standards (NBS, Washington, DC) Standard Reference Material (SRM) 1566 (oyster tissue) were also processed with samples. The precision (coefficient of variation) was 1.6% for Al and 1.2% for Fe determinations. The accuracy (relative error) of Fe values for SRM 1566 (mean ± SD = $188 \pm 2.2\ \mu g \cdot g^{-1}$) compared with the NBS certified value (mean ± CI = $195 \pm 34\ \mu g \cdot g^{-1}$) was 3.6%. Because of lack of a certified value, determinations for Al in SRM 1566 (mean ± SD = $135 \pm 2.2\ \mu g \cdot g^{-1}$) were compared with recent measurements made by instrumental neutron activation analysis (mean ± SD = $252 \pm 6\ \mu g \cdot g^{-1}$; Kratochvil et al. 1986). The relative difference was large (46%), indicating a possible underestimation of Al in the gills of parr. The high precision nevertheless indicated that

TABLE 1. Mean water chemistry of Nova Scotia rivers where pens of Atlantic salmon parr were located and of the hatchery (control) during autumn 1985.

	Medway River (N = 9)	Westfield River (N = 9)	Halfway Brook (N = 9)	Moose Pit Brook (N = 9)	Mersey Hatchery (N = 2–3)
pH[a]	5.2 *b*	5.0 *b*	4.7 *a*	4.6 *a*	5.4 *c*
Alkalinity (mg·L^{-1} as $CaCO_3$)	0.97 *d*	0.53 *c*	−0.04 *a*	−0.48 *b*	0.95 *cd*
Conductivity (mS·m^{-1} at 25°C)[a]	3.13 *a*	2.85 *a*	3.32 *a*	3.80 *b*	2.96 *a*
Na^+ (mg·L^{-1})	3.10 *b*	2.76 *a*	2.85 *a*	3.03 *ab*	3.08 *ab*
K^+ (mg·L^{-1})	0.33	0.35	0.38	0.39	0.26
Ca^{2+} (mg·L^{-1})	1.06 *b*	0.79 *a*	0.83 *a*	1.02 *b*	0.92 *ab*
Mg^{2+} (mg·L^{-1})	0.59 *bc*	0.49 *a*	0.50 *ab*	0.65 *c*	0.50 *ab*
Cl^- (mg·L^{-1})	4.5 *b*	3.9 *a*	3.9 *a*	4.5 *b*	4.7 *b*
SO_4^{2-} (mg·L^{-1})	2.5 *ab*	2.3 *a*	2.4 *a*	2.4 *a*	2.9 *b*
DOC (mg·L^{-1})	12.5 *a*	11.7 *a*	16.7 *b*	18.4 *b*	6.9 *a*
Al (µg·L^{-1})					
Total	228 *b*	261 *b*	331 *c*	245 *b*	164 *a*
Inorganic	21 *ab*	27 *bc*	37 *c*	15 *a*	36 *bc*

NOTE: Means followed by the same letters are not significantly different (Duncan's test, α = 0.05, performed when ANOVA probability <0.05).

[a] Average of mean weekly values.

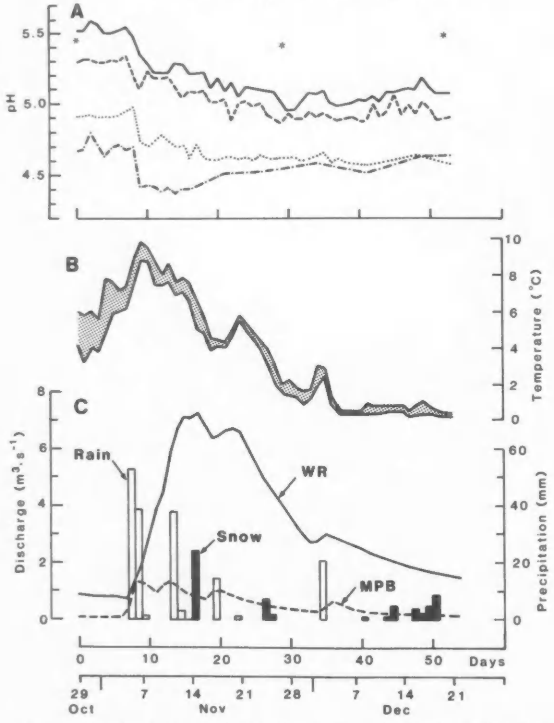

FIG. 2. (A) pH levels in the Medway River (—), Westfield River (---), Halfway Brook (···), Moose Pit Brook (-··-), and at the Mersey Hatchery (*); (B) range of mean daily water temperature for the four streams; (C) flow data for the Westfield River (WR) and Moose Pit Brook (MPB), and precipitation data for the Medway drainage (histogram) during exposure of Atlantic salmon parr in 1985. Flow data and climate summary from Environment Canada, Water Resources Branch, and Atmospheric Environment Service, respectively.

relative comparisons within our data sets are justified. As a further verification, Al in SRM 1566 was analyzed after ashing at a higher temperature (550°C) in platinum crucibles for 24 h, extraction with 2 mL of hot concentrated HNO_3, and dilution to 100 mL; duplicate analysis of Al by GFAA yielded 153 and 155 µg·g^{-1}, adding support to the accuracy of our method.

Survival curves for the censored survival data were estimated by the product-limit (Kaplan–Meier) method, and the equality of the survival distributions was tested by nonparametric rank tests (Mantel–Cox and Breslow statistics) using the BMDP statistical package (Benedetti et al. 1983). The comparison of observations among rivers (e.g. water and plasma chemistry, frequency of various behaviors, metal accumulation in gills) was made by tests (ANOVA, *t*-test) using the SPSS statistical package (SPSS Inc. 1986). Significant ANOVA were followed by a posteriori comparisons using Duncan's new multiple range test or the Tukey–Kramer procedure as appropriate based on sample size considerations (Kirk 1982).

Results

Water Chemistry

When parr were placed in the streams the pH was at maximum annual levels. One week later, the pH levels started to decline after the first major precipitation event of autumn (Fig. 2A, 2C). The pH levels decreased by about 0.3–0.5 unit during the next 3 wk, but remained different among the four streams, although the seasonal trends were similar. Changes in pH were most abrupt within 2–3 d of the first rainfall and more gradual thereafter.

Water temperature was similar at the four sites (±2°) and declined from a maximum of almost 10°C after the first rainfall to <1°C during the last 2 wk of the 54-d exposure period (Fig. 2B). Dissolved oxygen concentrations were always >90% of air saturation both inside and outside the pens at each site. Gran alkalinity was significantly different among the four

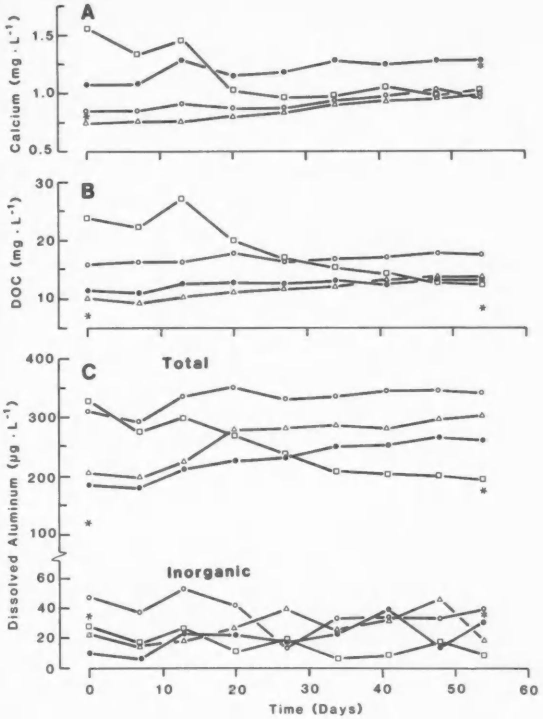

FIG. 3. Concentrations of (A) Ca, (B) DOC, and (C) total dissolved Al and inorganic Al in the Medway River (●), Westfield River (△), Halfway Brook (○), Moose Pit Brook (□), and at the Mersey Hatchery (∗) during exposure of Atlantic salmon parr starting October 29, 1985.

streams and was negative in the more acidic Halfway and Moose Pit brooks (Table 1). Concentrations of the major basic cations (Na^+, Ca^{2+}, Mg^{2+}) were on average significantly higher in Moose Pit Brook and the Medway River (the most and least acidic sites) than in the other two streams, and the dominant anion, Cl^-, was similarly different among streams (Table 1). Water conductivity levels were low at all sites (2.9–3.8 $mS \cdot m^{-1}$) as were Ca concentrations (0.8–1.1 $mg \cdot L^{-1}$). Calcium concentrations were initially highest in the more acidic Moose Pit Brook, but decreased after the first rainfall, whereas they increased in the other three streams (Fig. 3A). Dissolved organic carbon (DOC) concentrations were always high (>10 $mg \cdot L^{-1}$, Fig 3B) and were significantly higher in the more acidic headwater streams (Moose Pit and Halfway brooks) than in the Westfield and Medway rivers (Table 1). The seasonal trends for DOC were similar to those for Ca concentrations (Fig. 3A, 3B). Total dissolved Al concentrations were initially highest in the two most acidic streams but concentrations in Moose Pit Brook declined markedly, as DOC concentrations had, to the lowest levels by the end of the exposure in December (Fig. 3C). In contrast, Al concentrations in the other three streams increased gradually with decreasing pH levels and increasing DOC concentrations (Fig. 2A, 3B, 3C). Although total Al concentrations were high (200–350 $\mu g \cdot L^{-1}$), the concentrations of inorganic Al were always < 50 $\mu g \cdot L^{-1}$ in all four streams (Table 1; Fig. 3C).

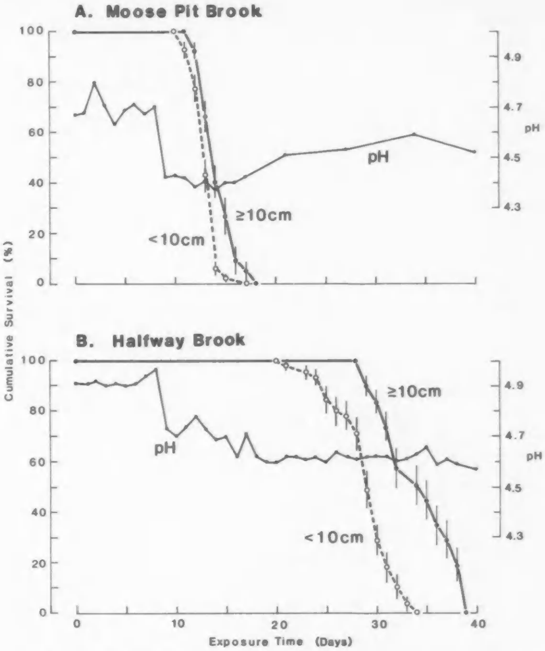

FIG. 4. Cumulative survival (±SE) of small Atlantic salmon parr (<10 cm) (○) and large parr (≥10 cm) (●) and pH levels in (A) Moose Pit Brook and (B) Halfway Brook during exposure starting October 29, 1985 ($N = 120$, the initial number of parr in each stream).

Lethal Responses

Survival curves indicate the lethality of conditions in Moose Pit and Halfway brooks where all fish died within 20 and 40 d, respectively (Fig. 4A, 4B). In contrast, there were no mortalities in the Westfield and Medway rivers where pH levels decreased by about 0.5 unit during the 54 d of exposure but never fell below pH 4.8 (Fig. 2A). Mortality in Moose Pit and Halfway brooks began only after the pH level had decreased below pH 4.7. Mortality was rapid, with most fish dying in a short time (5–10 d). The mean survival times were 13.9 d in Moose Pit Brook and 31.9 d in Halfway Brook, and the survival distributions were significantly different ($P < 0.0001$).

Survival curves of the two different size categories contained in each pen were significantly different ($P < 0.0002$, Fig 4A, 4B). The mean survival time of small parr (<10 cm) was less than that of large parr (≥ 10 cm) in Moose Pit Brook (13.2 vs. 14.4 d), and especially in Halfway Brook where small parr died on average 5 d earlier than large parr (29.1 vs. 34.4 d).

Physiological Responses

The physiological responses of parr to differences in water chemistry among streams included changes in plasma Na^+ and Cl^- concentrations and hematocrit (Fig. 5). Plasma Na^+ and Cl^- concentrations remained constant in the control parr from the hatchery. Concentrations decreased slightly (20–30 $mmol \cdot L^{-1}$) in parr after 28 d of exposure in the Medway and Westfield rivers, and were still decreasing at the end of the

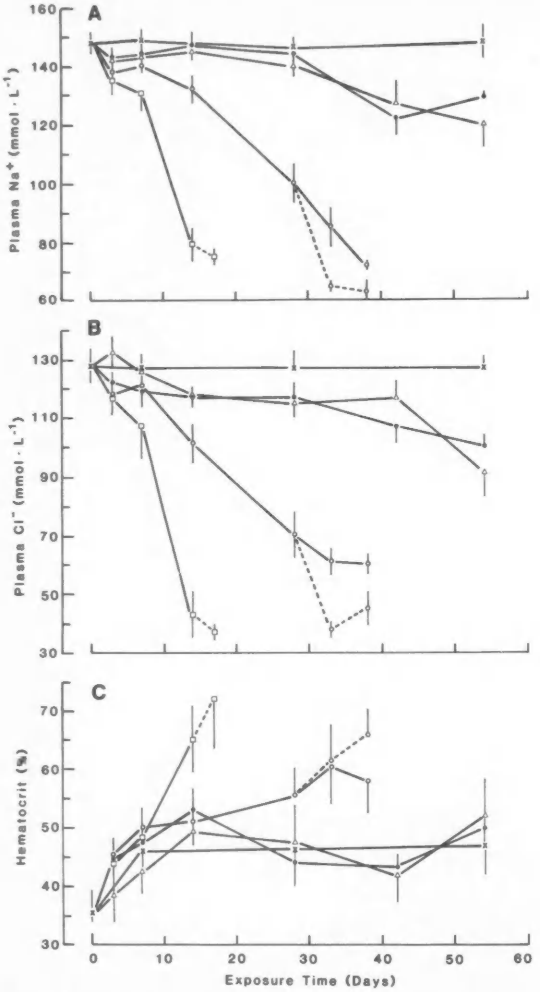

FIG. 5. Mean ± SD (sample sizes ≥8) (A) plasma [Na$^+$], (B) plasma [Cl$^-$], and (C) hematocrit determined in Atlantic salmon parr from the Mersey Hatchery (control) (×), Medway River (●), Westfield River (△), Halfway Brook (○), and Moose Pit Brook (□) during exposure starting October 29, 1985. Means (±SD, $N \geq 5$) for moribund fish in Moose Pit Brook and Halfway Brook are shown as separate samples (broken lines).

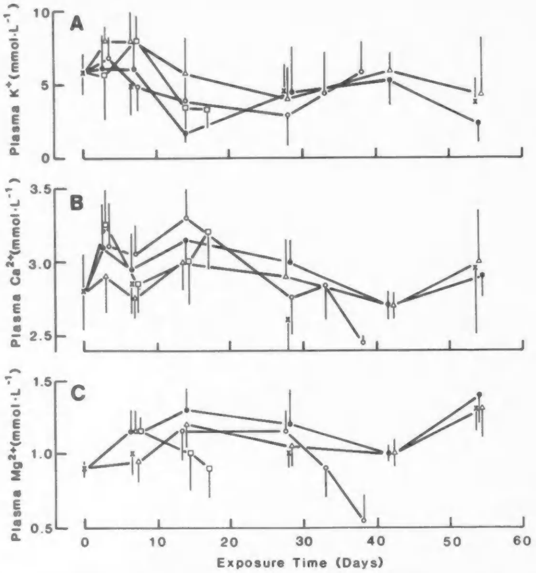

FIG. 6. Mean ± SD (sample sizes ≥8) plasma concentrations of (A) K$^+$, (B) Ca^{2+}, and (C) Mg^{2+} determined in Atlantic salmon parr from the Mersey Hatchery (control) (×), Medway River (●), Westfield River (△), Halfway Brook (○), and Moose Pit Brook (□) during exposure starting October 29, 1985.

study. In contrast, plasma Na$^+$ and Cl$^-$ in parr in Moose Pit Brook decreased by about 20 mmol·L^{-1} during the first 7 d and by more than 50 mmol·L^{-1} during the next 7 d as fish were dying. The parr in Halfway Brook initially (3 d) lost similar amounts of plasma Na$^+$ and Cl$^-$ to those in Moose Pit Brook, but electrolyte losses then continued at a more gradual rate from days 7 to 28, after which most deaths occurred. Plasma Na$^+$ and Cl$^-$ levels from moribund fish were significantly different from those of other parr (t-tests, $P < 0.05$, Fig 5A, 5B). Parr became moribund and usually died within 24 h when plasma Na$^+$ decreased below 80 mmol·L^{-1} and Cl$^-$ below about 60 mmol·L^{-1}. Losses of plasma Na$^+$ and Cl$^-$ were large (>50% of control values) before death, and the ratio of Na$^+$:Cl$^-$ loss was 1:1.23 in parr from Moose Pit Brook after 14 d and 1:1.21 in parr from Halfway Brook after 28 d, indicating a larger loss of Cl$^-$ than Na$^+$. Although plasma Na$^+$ and Cl$^-$ losses were relatively minor and no deaths occurred in the Westfield and Medway rivers, the ratios of Na$^+$:Cl$^-$ loss were 1:1.32 and 1:1.47, respectively. Comparisons of the ratios over time for parr in Halfway Brook indicate that Cl$^-$ is initially lost is greater amounts than Na$^+$ (Na$^+$:Cl$^-$ loss of 1:1.69 after 14 d and 1:1.21 after 28 d), but that Na$^+$ losses are larger later in the exposure (Na$^+$:Cl$^-$ loss of 1:1.06 after 33 d and 1.12:1 after 38 d in non-moribund parr).

Hematocrit increased similarly in all groups of parr including controls during the first 7 d (from 35 to 45–50%, Fig. 5C). Thereafter, hematocrit increased rapidly in parr in Moose Pit Brook and more gradually in those in Halfway Brook. Hematocrits were >60% in moribund parr.

Differences in plasma Na$^+$ and Cl$^-$ concentrations and hematocrit between the two size categories of parr (<10 and ⩾10 cm) were mostly minimal and nonsignificant (t-tests, $P > 0.05$) in each stream regardless of the size-related differences in survival time observed in Moose Pit and Halfway brooks. Differences were probably not apparent because the time interval between sampling periods was greater than the difference in survival time between size categories.

Plasma K$^+$, Ca^{2+}, and Mg^{2+} concentrations in parr usually did not differ significantly among streams or from controls (ANOVA, $P < 0.05$, Fig. 6), and there were no significant differences between concentrations in moribund and other parr in Moose Pit and Halfway brooks (t-tests, $P > 0.05$). Sample variances for K$^+$ and Ca^{2+} were large and masked any trends. Plasma Mg^{2+} concentrations in parr from Moose Pit and Halfway brooks decreased in the days before death, but the decline was significant (>50% loss) only in parr in Halfway Brook.

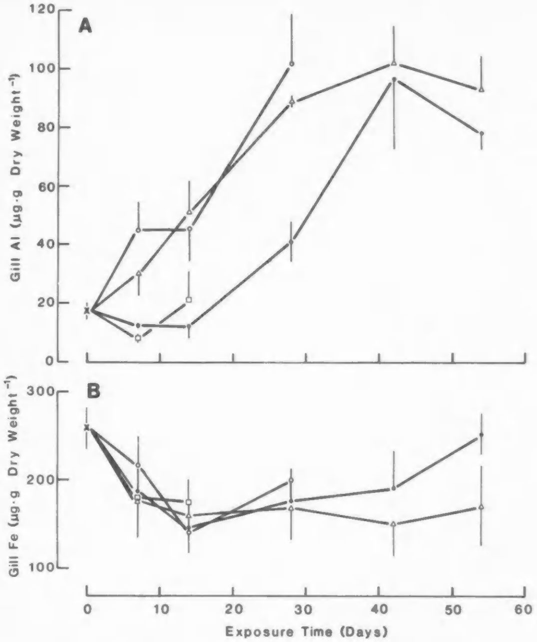

FIG. 7. Mean ± SD (sample sizes ≥5) concentrations of (A) Al and (B) Fe in the gills of Atlantic salmon parr from the Mersey Hatchery (control) (×), Medway River (●), Westfield River (△), Halfway Brook (○), and Moose Pit Brook (□) during exposure starting October 29, 1985.

Gill Al Accumulation

Concentrations of Al in the gills of parr increased from <20 µg·g dry weight^{-1} to about 100 µg·g dry weight^{-1} during the period of exposure in the Medway and Westfield rivers and Halfway Brook (Fig. 7A). In Moose Pit Brook, all fish died before any significant accumulation of Al in gills occurred (mean ± SD = 21 ± 10 µg·g dry weight $^{-1}$), and concentrations were similar to those in parr of the Medway River at that time. Accumulation of Al in the gills of parr in the Medway River was delayed during the first 14 d and was significantly lower than in those of parr in the Westfield River and Halfway Brook (ANOVA, $P < 0.01$; Duncan's test, $\alpha = 0.01$) until after 40 d of exposure when Al concentrations appeared to stabilize between 80 and 100 µg·g dry weight^{-1}. Aluminum concentrations in the gills of parr in Halfway Brook were at this maximum level (102 ± 17 µg·g dry weight^{-1}) when mortality peaked. However, no parr in the Medway and Westfield rivers died, although levels of Al accumulation in the gills of parr were similar to those of parr in Halfway Brook.

Concentrations of Fe in the gills of parr initially decreased from a maximum in fish from the hatchery (mean ± SD = 257 ± 26 µg·g dry weight^{-1}) to an average of about 150 µg·g dry weight^{-1} during the first 14 d after transfer to the four streams (Fig. 7B). Then Fe concentrations remained relatively constant at this basal level until the last sampling date. There were no significant differences in Fe concentrations in the gills of parr among the four streams at any time (ANOVA, $P > 0.10$), except between parr in the Medway and Westfield rivers at the end of exposure (54 d; t-test, $P < 0.01$).

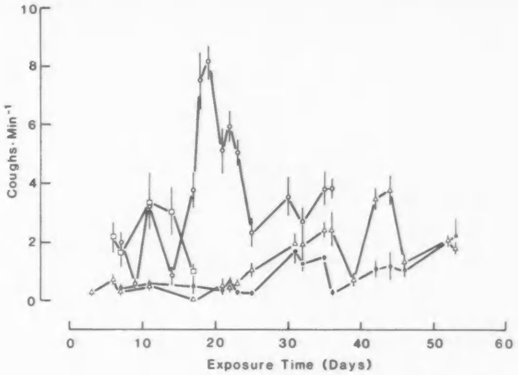

FIG. 8. Mean ± SE (sample sizes =10) frequency of coughing by Atlantic salmon parr in the Medway River (●), Westfield River (△), Halfway Brook (○), and Moose Pit Brook (□) during exposure starting October 29, 1985.

Behavioral Responses

Some of the behaviors of parr were apparently altered in response to changes in water chemistry. The frequencies of breathing and coughing by parr were significantly different among the streams (Table 2). Breathing rate was greater for parr in Halfway Brook than for parr in the Westfield and Medway rivers, and the difference was probably pH related because temperature, dissolved oxygen, and water velocity inside the pens were essentially the same at each site. Difficulties in accurately enumerating the subtle breathing activity resulted in small sample sizes and insufficient data to assess changes over time at any of the sites.

Coughing frequency was higher by parr in Moose Pit and Halfway brooks than in the other two less acidic streams (Table 2). There were no apparent differences in suspended particulate matter in the water which could account for the large differences in the response among streams. The difference was established in the first week of exposure and the rate of coughing increased markedly as pH levels decreased, especially by parr in Halfway Brook, until parr started to die (Fig. 8). The frequency of coughing then decreased in both streams as parr became moribund. In the Westfield and Medway rivers, coughing by parr increased gradually to more than twice the initial values as pH levels decreased to about pH 5.0 and was still rising at the end of the study (Fig. 8).

The frequency of yawning by parr was low compared with coughing, and although the ANOVA indicated that differences among sites were significant ($P < 0.05$), no two sites were found to differ in a posteriori comparisons (Table 2). Yawning rates decreased over time in all four streams and were almost nil for parr in Moose Pit and Halfway brooks before death.

Aggressive behavior among parr was observed to be infrequent at all sites (less than one aggressive act of any type per 40 min of observation of individual fish) and chafing was even less frequent. The frequencies of any of the recorded behaviors usually did not differ significantly between the two size categories of parr (<10 and ≥10 cm) at any time in each stream (t-tests, $P > 0.05$).

The relative availability of drifting food items passing through the pen at the different sites was not determined, but the parr fed on drift at a similar frequency (0.1–0.2 responses·

TABLE 2. Mean behavioral responses by Atlantic salmon parr held in pens in four acidic rivers of Nova Scotia during autumn 1985; SD in parentheses under mean, number of fish observed (*n*), and number of observation periods (*N*).

	Medway River $(N = 15)^a$	Westfield River $(N = 19)^a$	Halfway Brook $(N = 16)^a$	Moose Pit Brook $(N = 5)^a$
Breathing				
(frequency·min^{-1})	42.7 *a*	40.4 *a*	51.0 *b*	
	(6.1)	(7.3)	(9.6)	—
	(*n* = 10)	(*n* = 14)	(*n* = 34)	
Coughing				
(frequency·min^{-1})	0.75 *a*	1.39 *b*	3.90 *d*	2.40 *c*
	(0.78)	(1.43)	(2.77)	(2.32)
	(*n* = 120)	(*n* = 185)	(*n* = 153)	(*n* = 42)
Yawning				
(frequency·min^{-1})	0.27 *a*	0.39 *a*	0.29 *a*	0.41 *a*
	(0.31)	(0.47)	(0.39)	(0.47)
	(*n* = 120)	(*n* = 185)	(*n* = 153)	(*n* = 43)
Feeding responses				
Pellet feeding				
(% of food taken)	36.9 *b*	39.1 *b*	1.3 *a*	
	(39.8)	(32.7)	(2.7)	—
	(*N* = 10)	(*N* = 12)	(*N* = 13)	
Feeding intensity				
(0–3 scale)^b	2.63 *b*	2.21 *b*	0.92 *a*	
	(0.67)	(0.70)	(0.86)	—
	(*N* = 11)	(*N* = 14)	(*N* = 13)	

NOTE: Means followed by the same letters are not significantly different (Tukey–Kramer test, $\alpha = 0.05$, performed when ANOVA probability <0.05).

[a] *N* = number of observation periods for each behavior unless indicated otherwise.

[b] Feeding intensity: no feeding (0), sluggish feeding (1), moderate (steady) feeding (2), and vigorous feeding (3) on pellets.

min^{-1}) at all sites, except Moose Pit Brook where all feeding ceased after several days of exposure. The percentage of pellets taken by parr in the post-observation feeding period was highly variable over time, but was consistently greater in the Medway and Westfield rivers than in the more acidic Halfway Brook where the quantity of food consumed was low (Table 2). The feeding intensity of parr in Halfway Brook was also significantly lower than that of parr in the Medway and Westfield rivers (Table 2). The feeding intensity in Halfway Brook declined sharply to near zero after 20 d when parr started to die. In the Medway and Westfield rivers, feeding intensity decreased from vigorous to moderate and then to sluggish, but only during the last few weeks of the exposure period. Low water temperature (0.1–1.2°C, Fig 2B) may have been a factor causing the decrease at that time.

Discussion

Juvenile Atlantic salmon mortalities in the acidic streams of Nova Scotia occurred only when pH levels decreased below pH 4.7 for at least several days. Deaths occurred rapidly when pH levels remained slightly below pH 4.7 (Halfway Brook) and were still more rapid at pH 4.4 (Moose Pit Brook). The resistance of parr at lethal pH levels in the streams was size dependent with small parr dying more rapidly than large parr. Increased resistance of rainbow trout (*Salmo gairdneri*) to acidity based on size has previously been postulated by Giles et al. (1984) from indirect evidence. Similarly, the positive correlation between survival time at pH 3.6 and age of rainbow trout observed by Lloyd and Jordan (1964) was presumably a function of size.

The magnitude and rapidity of plasma Na^+ and Cl^- losses and related increases in hematocrit in parr of the different streams were distinctly related to differences in pH among the four streams, representing effects ranging from sublethal to acutely lethal. Comparisons between moribund and apparently normal parr at low pH levels indicate that incipient lethal effects occur when plasma Na^+ and Cl^- concentrations have decreased below about 80 and 60 mmol·L^{-1}, respectively, and hematocrit has increased beyond 60%. Our observations are generally similar to those of Giles et al. (1984) for rainbow trout in a series of laboratory bioassays. Physiological effects similar to those in our study were also previously observed in juvenile salmon residing in acidic streams of Nova Scotia, but the responses were not as pronounced and were indicative of sublethal effects (Lacroix 1985b).

Ionoregulatory failure in fish has been emphasized as an important toxic mechanism and as the single most adverse sublethal effect caused by reduced pH levels (see reviews by Fromm 1980; Spry et al. 1981; Leivestad 1982; Wood and McDonald 1982; McDonald 1983a). The severe ionic imbalance caused by this ionoregulatory failure was probably responsible for the death of parr at low pH levels in the two most acid streams. The losses of plasma Na^+ and Cl^- at lethal pH levels were massive and almost equimolar at death, although Cl^- was initially lost in greater amounts than Na^+. Similarly, the sublethal effects at pH levels of about 4.8–5.2 resulted in larger proportional losses of Cl^- than Na^+ from plasma. These effects parallel those described for laboratory tests at low pH levels and low Ca concentrations (McDonald et al. 1980, 1983; Wood and McDonald 1982; McDonald 1983a, 1983b). The lethal and sublethal physiological responses and the differential effects on Na^+ and Cl^- observed in juvenile salmon thus lend further support to the laboratory models for acid exposure in soft water.

In nature, where acidity and Al invariably increase together in soft water, identification of the lethal factor is often complex, especially since both factors cause similar physiological effects which may be additive (see reviews by Spry et al. 1981; Baker 1982; Leivestad 1982). The presence of inorganic Al increases the lethal effects at low pH (4.5) by increasing the rate of ion loss compared with the response to acid alone (cf. Wood and McDonald 1982; Neville 1985). Ionic depletion in fish in response to Al is very rapid, usually within hours at lethal concentrations (Fivelstad and Leivestad 1984; Rosseland and Skogheim 1984), whereas the reaction in response to low pH usually occurs only after days or weeks of exposure (Wood and McDonald 1982). Therefore, the ionic depletion in parr in our study was typical of the soft water ionic response to low pH (4.0–4.5) of salmonids described by Wood and McDonald (1982), viz. large, nearly equimolar losses of Na^+ and Cl^- (about 40 $mmol \cdot L^{-1}$) over 5 d or more of exposure. The rate of ion loss differed from that usually associated with Al toxicity, indicating that H^+ was the most likely lethal factor. Furthermore, regardless of total Al concentrations in the streams, inorganic Al concentrations were $<50\ \mu g \cdot L^{-1}$ and below concentrations generally considered toxic to fish (Burrows 1977; Baker and Schofield 1982; Muniz and Leivestad 1980; Rosseland and Skogheim 1984; Fivelstad and Leivestad 1984; Neville 1985).

Aluminum accumulated in the gills of parr, but the lethal effects were apparently independent of this accumulation as evidenced by parr mortality in Moose Pit Brook in the absence of Al accumulation in gills and by parr mortality in Halfway Brook where the extent of Al accumulation in gills was identical to that in parr in the Westfield and Medway rivers. Had Al been toxic to parr in our study, the maximum effect should have occurred in the Westfield and Medway rivers because Al is most toxic at pH 5.0–5.5 (Baker and Schofield 1982; Muniz and Leivestad 1980; Fivelstad and Leivestad 1984), yet there were no deaths. Furthermore, because of the relationship between Al and organic matter (Lacroix and Kan 1986), inorganic Al concentrations were low in the four streams and lowest in Moose Pit Brook where conditions were most toxic. In contrast with Al, Fe concentrations in the gills of parr decreased to a new equilibrium. The high Fe concentrations reflect the presence of Fe in blood and thus in gills and are considered to be representative of the gills of healthy fish (Andersson and Nyberg 1984).

Behavioral changes occurred in parr in response to the lethal and sublethal conditions in the four acidic streams. However, as noted by Sprague (1971), the significance of these changes in nature is uncertain. Increased breathing and coughing frequencies and decreased feeding responses at the lower pH levels were the most clear-cut changes. The rate of coughing by fishes is affected by a wide range of toxicants and is related both to sublethal concentration and to time of exposure (Schaumberg et al. 1967). However, Hargis (1976) showed that the respiratory–cough response was pH dependent in rainbow trout, and Giles et al. (1984) recorded significant increases in breathing rates of rainbow trout at $pH < 4.9$. These responses, as well as cessation of feeding, provided corroborative evidence of the stress on parr; however, they are such general responses to pollutant-caused stress or to any deterioration in health that they probably cannot be used independently as definitive indicators of acid stress.

The lethal effects of low pH levels on parr in Moose Pit and Halfway brooks may explain why no juvenile salmon were found in those streams and why stocking attempts have been unsuccessful (G. L. Lacroix, unpubl. data). Land use surveys of the Westfield River basin, inclusive of Halfway and Moose Pit brooks, indicate no alternative factors or sources of pollution that could be the cause of the response in fish observed during autumn (DeGraeve and Peterson 1982). The area is sparsely inhabited, agriculture is unimportant (<100 ha), and most of the land is forested. There is intensive forest harvesting, but chemical pesticides are not used. In the Medway and Westfield rivers, pH levels did not decrease enough to cause mortalities of parr during 1985, but conditions during previous years have been acidic enough at times ($pH < 4.7$) to cause substantial losses to the native parr population, especially in the Westfield River (G. L. Lacroix, unpubl. data). The continued presence of juvenile salmon in that river, albeit in reduced numbers, is due largely to the limited reproductive success of returning salmon from an enhancement program in the Medway River system. Our study indicates that Al is not responsible for the lethal effects associated with the acidification of the "brown waters" characteristic of a large number of the salmon rivers in Nova Scotia. Increased H^+ concentration is considered to be the primary lethal factor affecting juvenile salmon stocks in these rivers.

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Influence of Interannual Variations in Winter Sea Temperature on Fecundity and Egg Size in Pacific Herring (*Clupea harengus pallasi*)

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Tanasichuk, R. W., and D. M. Ware. 1987. Influence of interannual variations in winter sea temperature on fecundity and egg size in Pacific herring (*Clupea harengus pallasi*). Can. J. Fish. Aquat. Sci. 44: 1485–1495.

Data for 2937 fish, collected from seven locations over five years, were analysed to evaluate the effects of sea temperature and stock biomass on size-specific ovary weight and fecundity at spawning. Ovary weight did not vary significantly between years or locations. Size-specific fecundity was higher in 1983, when coastal waters were abnormally warm because of a strong El Niño – Southern Oscillation event. The effect of location was equivocal: one stock that overwintered in warm water tended to have a higher fecundity. Mean sea temperature between 60 and 90 d before spawning (in spring) best accounted for variations in size-specific fecundity. Temperature may influence fecundity by regulating gonadotropin concentration and consequently pre-ovulatory atresia. We hypothesize that the trade-off between fecundity and egg size is adaptive. A theoretical analysis of the early life history of Pacific herring suggests that, to maximize survival to metamorphosis, egg size should decrease and fecundity increase with temperature when the larval growth rate Q_{10} is less than the mortality rate Q_{10}. Our model seems to explain the differences in egg size between recruit and repeat spawners, and between stocks of Atlantic herring that spawn in different seasons.

Les auteurs ont analysé les données recueillies sur 2 937 poissons capturés à sept endroits au cours d'une période de 5 ans afin d'évaluer les incidences de la température de la mer et de la biomasse des effectifs sur le poids des ovaires selon la taille et la fécondité au moment du frai. Le poids des ovaires n'a pas nettement varié d'une année à l'autre ou d'un endroit à l'autre. La fécondité selon la taille était plus élevée en 1983 quand les eaux côtières sont devenues anormalement chaudes à cause d'un fort déplacement du courant El Niño vers le sud. L'incidence de l'endroit était ambiguë : un stock qui a passé l'hiver dans les eaux chaudes montrait une fécondité plus élevée. La température moyenne de la mer de 60 à 90 jours avant le frai (au printemps) traduisait mieux les variations de la fécondité selon la taille. La température peut influer sur la fécondité en réglant la concentration de gonadotrophine et donc sur l'atrésie préovulatoire. Les auteurs formulent l'hypothèse que le compromis entre la fécondité et la taille des oeufs est un mécanisme adaptif. Une analyse théorique du cycle vital primordial du hareng du Pacifique porte à croire que la taille des oeufs devrait diminuer et que la fécondité devrait augmenter en fonction de la température quand le taux de croissance des larves Q_{10} est inférieur au taux de mortalité Q_{10} afin de maximiser la survie jusqu'à la métamorphose. Le modèle présenté semble expliquer les différences entre la taille des oeufs des recrues et des géniteurs multifrai et aussi entre les stocks de hareng de l'Atlantique qui fraient à différentes saisons.

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Sea temperatures along the northwest coast of North America were unusually high in 1982–83 because of a very strong El Niño – Southern Oscillation (ENSO) event. A pronounced and anomalous warming of surface and subsurface waters began in the eastern equatorial Pacific in late fall 1982 and reached the Gulf of Alaska by January 1983 (R. Thomson, Institute of Ocean Sciences, Sidney, B.C., pers. comm.). Water temperatures off the Oregon coast in January and April 1983 were 1.5–2.0°C higher at 10 m depth to a distance of 50 km offshore with weaker anomalies extending seaward to a maximum of 200 km. Temperatures at 100 m depth were 1.5°C higher out to 200 km. Water temperature below 200 m did not appear to be influenced. Sea surface temperature anomalies along the British Columbia coast increased abruptly during December 1982 – January 1983, rose by a maximum of 2.5°C between March and May, and then began to decline in June (Tabata 1985).

Sea temperature may be responsible for some differences in egg size and fecundity (total egg number per female) among various stocks of Atlantic (*Clupea harengus harengus*) and Pacific (*Clupea harengus pallasi*) herring. Hempel and Blaxter (1967) described a seasonal variation in dry weight of ripe Atlantic herring eggs which implies that egg size is negatively correlated with water temperature between stocks. There are numerous reports that size-specific fecundity (fecundity at a specified body size) is higher in Atlantic herring stocks spawning in warmer water (e.g. Baxter 1959; Parrish and Saville 1965; Cushing 1967; Hodder 1972; Messieh 1976) and in Pacific herring spawning at lower latitudes (Katz 1947; Nikolskii 1969; Paulson and Smith 1975). However, it is

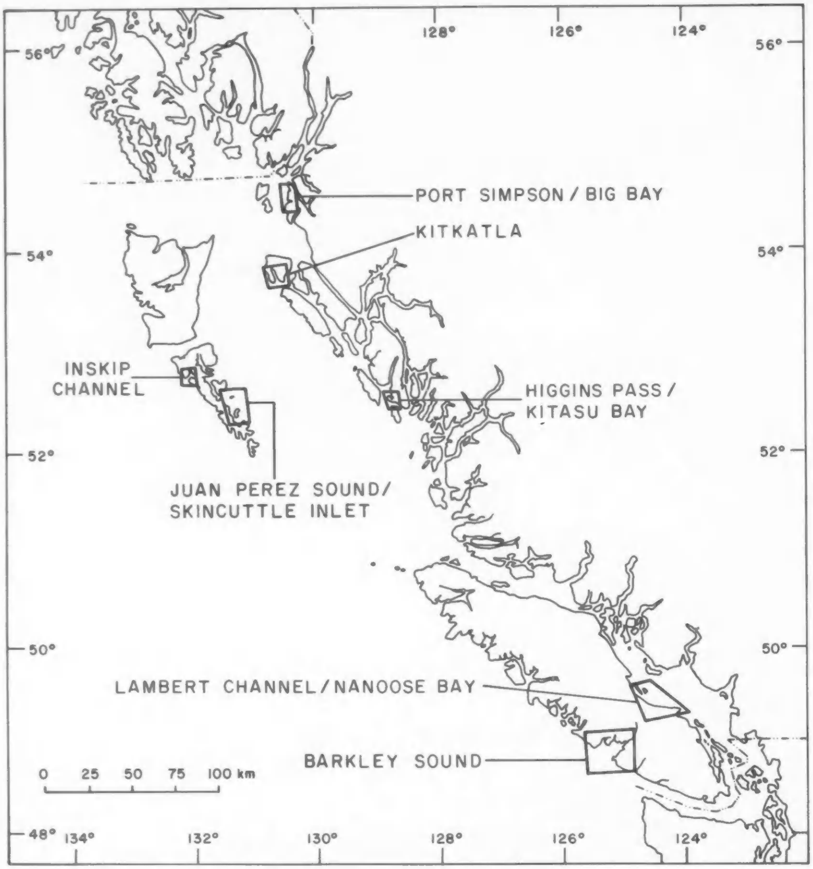

FIG. 1. Herring fecundity sampling locations.

unclear whether temperature affects fecundity or egg weight and if there is also an effect on the total reproductive output (i.e. ripe ovary weight). These variables are interrelated by the following simple expression: egg size = ripe ovary weight/fecundity.

Although variations in the reproductive characteristics of herring stocks spawning at different temperatures are well documented, no one has examined the influence of interannual temperature changes on ovary weight, fecundity, and egg size of a specific population. Hempel (1965) argued that stock-related differences in egg size of Atlantic herring are selected to maximize larval survival in response to temperature-induced variations in the food supply and predation pressure. Is it possible that differences in fecundity and egg size occurring between herring stocks spawning at different temperatures might also take place within a single stock exposed to interannual temperature variations? Presumably, the ability to adjust fecundity (to compensate for some future expectation of the predation rate) or larval size (to better suit feeding conditions during the early life history) would be selected for in preference to some inflexible strategy, which produces a fixed number of larvae that are invariably constant in size and, hence, ill-adapted in some situations.

The objective of this study was to take advantage of the recent, strong ENSO event, which occurred during a routine fecundity monitoring program, by examining the impact of interannual differences in sea temperature on the reproductive characteristics of British Columbia Pacific herring stocks. Specifically, we were interested in possible shifts in fecundity and egg size and whether or not these changes might be adaptive.

Fecundity

Materials and Methods

Fecundity data for herring captured in 1974 (Hourston et al. 1981a), 1980 (D. Hay, unpubl. data), and 1983–85 (R. Tanasichuk, unpubl. data) were analysed. All fish were collected in spring (March–April), within 25 d of the beginning of significant spawning and, consequently, would have been at a minimum of Stage IV maturity based on a modified version of the Hjort scale (Hay 1985). Samples were taken from purse-seine catches made near major spawning locations (Fig. 1).

Samples were frozen and shipped to the Pacific Biological Station for processing. All fish collected in 1974 and 1980 were examined. However, collections made between 1983 and 1985 were stratified by length intervals that approximated successive age groups. These intervals were estimated from biological samples collected in each locality during the previous year.

TABLE 1. Intercepts (i) and error mean squares (EMS) for three predictive regression equations: (1) ln fecundity − ln total weight; (2) ln ripe ovary weight − ln total weight; and (3) ln fecundity − ln standard length, respectively, for subgroups of British Columbia herring. Missing intercepts are for subgroups from which samples were collected too early to provide ripe ovary weight estimates. Common slopes (1.05, 1.24, and 3.27) for these three respective regression equations were estimated with ANCOVA. The EMS is used with the equation Bagenal and Braum (1978) provided to correct for the bias in the anti-logarithms of estimates made from logarithmic regression equations.

	1974		1980		1983		1984		1985	
Location	*i*	EMS	*i*	EMS	*i*	EMS	*i*	EMS	*i*	EMS
Lambert Channel − Nanoose Bay	5.00	0.02	5.04	0.03	5.17	0.02	5.07	0.01	5.05	0.01
	−2.51	0.01	−2.51	0.01	−2.49	0.01	−2.53	0.01	−2.47	0.01
	−7.50	0.03	−7.30	0.05	−7.12	0.03	−7.29	0.02	−7.24	0.02
Barkley Sound	5.10	0.02	5.00	0.02	5.17	0.02	5.06	0.03	5.13	0.01
	−2.45	0.01	—	—	−2.43	0.01	−2.41	0.02	−2.41	0.01
	−7.26	0.04	−7.26	0.04	−7.17	0.03	−7.28	0.06	−7.12	0.02
Kitasu Bay Higgins Pass	5.00	0.02	4.98	0.03	5.19	0.02	5.13	0.02	5.12	0.01
	−2.45	0.01	−2.50	0.01	—	—	—	—	—	—
	−7.45	0.02	−7.29	0.04	−7.15	0.02	−7.24	0.03	−7.16	0.02
Kitkatla	4.85	0.01	4.80	0.15	5.18	0.01	4.95	0.03	5.09	0.01
	—	—	—	—	−2.41	0.01	—	—	—	—
	−7.55	0.02	−7.48	0.14	−7.10	0.02	−7.40	0.03	−7.24	0.02
Port Simpson − Big Bay	5.01	0.02	4.97	0.01	5.15	0.01	4.99	0.02	5.00	0.02
	−2.52	0.01	−2.51	0.01	−2.44	0.01	−2.39	0.01	−2.44	0.01
	−7.37	0.03	−7.33	0.03	−7.12	0.01	−7.33	0.04	−7.27	0.03
Juan Perez Sound − Skincuttle Inlet	5.00	0.02	4.98	0.02	5.09	0.01	5.07	0.02	5.06	0.01
	−2.47	0.01	−2.49	0.01	—	—	−2.39	0.01	—	—
	−7.39	0.03	−7.29	0.03	−7.14	0.02	−7.32	0.03	−7.15	0.02
Inskip Channel	5.07	0.02	5.01	0.01	5.11	0.01	5.01	0.02	5.06	0.02
	−2.38	0.01	—	—	—	—	−2.45	0.01	—	—
	−7.25	0.02	−7.27	0.02	−7.15	0.01	−7.28	0.03	−7.21	0.03

Whenever possible, a maximum of 10 females were selected randomly from each length interval. Routine biological sampling consisted of measuring standard length to the nearest 1 mm, total wet weight to the nearest 1 g, and ovary wet weight to the nearest 1 g in 1974, 0.1 g in 1980, and 0.01 g between 1983 and 1985. In addition, three scales were removed from beneath the left pectoral fin for age determination. Fish extruding eggs were not sampled. In 1974 and 1980, entire ovaries were preserved in Gilson's fluid (Simpson 1951) for fecundity determinations. In 1983−85, the posterior three fourths of both ovaries were stored in Gilson's fluid for fecundity estimates; the anterior portions were frozen for calorimetric analysis.

Fecundity was estimated gravimetrically (Hourston et al. 1981b) for the 1974 and 1980 samples and with a photoelectric fish egg counter (Tanasichuk et al. 1985) for herring collected between 1983 and 1985. Sample preparation for both methods was essentially the same. The ovaries from each fish were stored for about 6 wk in a labelled 250-mL jar containing Gilson's fluid. Ovaries were sieved to break up egg clusters and to remove connective tissue fragments. The eggs were returned to the jars and stored in 10% formalin (1974 and 1980) or Gilson's fluid (1983−85). Fecundity estimates were obtained by multiplying eggs per gram in the counted subsample by the ovary wet weight. Tanasichuk et al. (1985) found that there were no significant differences ($p = 0.12$) between fecundity estimates made gravimetrically and with the photoelectric counter.

Data analysis began with identifying, and then excluding, herring which had apparently lost eggs at, or after, capture. Linear regressions using log-transformed values of ovary weight and total weight best described the relationship between these variables and were calculated for each year−location combination (subgroup). Outliers, i.e. those ovary weights where the Studentized residuals were less than −2, were assumed to be from fish which had extruded eggs — these fish were excluded from all subsequent analyses.

Fecundity and ripe ovary weight for the grand mean total weight (126 g) and fecundity for the grand mean standard length (209 mm) were estimated for each subgroup from fitted linear regression equations. Equations for ripe ovary weight could not be developed for all subgroups because some samples were collected more than 4 d prior to spawning, before the ovaries were completely mature. We found that the fecundity − total weight, ripe ovary weight − total weight, and fecundity − standard length relationships were best described by power curves (i.e. log-transformed values for the dependent and independent variables). Results of GT-2 tests (Sokal and Rohlf 1981) indicated that the slopes of the equations for all subgroups within each regression category (i.e. fecundity − total weight, ripe ovary weight − total weight, and fecundity − standard length) did not differ significantly ($p > 0.05$). Therefore, we used an analysis of covariance to calculate a common regression slope for each category and intercept for each year−location combination (Table 1). Ovary weight and fecundity at the grand mean weight and at the grand mean length, respectively, were then estimated for each subgroup. The bias in the antilogarithms of estimates derived from the

TABLE 2. Estimated fecundity, ripe ovary wet weight (g), mean egg wet weight at spawning (mg), mean surface sea temperature (°C) between 60 and 90 d before spawning, and biomass indices. Fecundity and ovary weight estimates are for the grand mean weight of fish (126 g) using the common regression slope and the intercept for each subgroup calculated for ln fecundity − ln total weight or ln ovary weight − ln total weight regressions from ANCOVA. Fecundity and ovary weight estimates were corrected using the method described by Bagenal and Braum (1978). Mean egg weight = ovary weight × 1000/fecundity. Biomass index is standardized relative to the 1974 biomass.

Parameter	1974	1980	1983	1984	1985
			Lambert Channel − Nanoose Bay		
Fecundity	24 414	25 668	28 929	25 884	25 369
Ovary weight	33.16	33.15	33.83	32.50	34.51
n	262	23	56	94	57
Egg weight	1.36	1.29	1.17	1.26	1.36
Sea temperature	7.3	7.4	8.1	7.5	7.2
Winter biomass index	1.00	1.70	1.50	0.84	0.49
Summer biomass index	1.00	1.62	1.33	0.88	0.60
			Barkley Sound		
Fecundity	26 982	24 402	28 929	26 212	27 482
Ovary weight	35.21	—	35.92	37.06	36.65
n	316	40	59	65	57
Egg weight	1.30	—	1.24	1.41	1.33
Sea temperature	7.8	7.5	8.0	7.5	7.0
Winter biomass index	1.00	1.48	1.05	0.94	0.77
Summer biomass index	1.00	1.62	1.33	0.88	0.60
			Kitasu Bay − Higgins Pass		
Fecundity	24 408	24 201	29 506	27 797	27 210
Ovary weight	35.21	33.49	—	—	—
n	70	101	35	72	76
Egg weight	1.44	1.38	—	—	—
Sea temperature	6.2	6.8	7.4	7.8	6.8
Winter biomass index	1.00	1.06	1.58	1.21	0.91
Summer biomass index	1.00	1.32	1.74	1.41	1.31
			Kitkatla		
Fecundity	20 765	23 172	28 892	23 473	26 405
Ovary weight	—	—	36.65	—	—
n	24	97	61	39	66
Egg weight	—	—	1.27	—	—
Sea temperature	6.3	7.2	7.8	7.8	7.0
Winter biomass index	1.00	2.62	2.55	2.37	3.31
Summer biomass index	1.00	1.32	1.74	1.41	1.31
			Port Simpson − Big Bay		
Fecundity	24 654	23 420	28 035	24 165	24 402
Ovary weight	32.83	33.16	35.56	37.39	35.56
n	80	86	39	64	40
Egg weight	1.33	1.42	1.27	1.55	1.46
Sea temperature	5.6	6.5	7.6	7.0	7.0
Winter biomass index	1.00	0.78	1.26	2.13	3.59
Summer biomass index	1.00	2.22	2.18	2.78	3.20
			Juan Perez Sound − Skincuttle Inlet		
Fecundity	24 415	23 929	26 407	26 176	25 625
Ovary weight	34.52	33.83	—	37.39	—
n	349	156	105	58	67
Egg weight	1.41	1.41	—	1.43	—
Sea temperature	5.6	6.5	7.6	7.1	7.0
Winter biomass index	1.00	3.26	2.84	3.24	2.92
Summer biomass index	1.00	2.32	2.28	2.67	3.23

TABLE 2. (*Concluded*)

Parameter	1974	1980	1983	1984	1985
			Inskip Channel[a]		
Fecundity	26 165	24 373	26 938	24 644	25 917
Ovary weight	37.75	—	—	35.20	—
n	28	46	52	33	64
Egg weight	1.44	—	—	1.43	—

[a] Insufficient information on overwintering locations.

log-transformed regression equations was corrected using the method recommended by Bagenal and Braum (1978).

The influence of sea temperature on herring reproductive characteristics was examined using reported mean monthly sea surface temperatures and estimates derived from them. Data relevant to each herring stock were selected from lighthouse stations that were closest to summer feeding and overwintering areas identified by tagging studies (C. Haegele, Department of Fisheries and Oceans, Nanaimo, B.C., pers. comm.) and the spawning grounds where herring tend to aggregate in the last month before spawning. Temperature estimates were not made for April or November when herring tend to be migrating to feeding and overwintering areas, respectively. Winter temperatures were estimated for each 30-d period over the last 90 d before spawning (generally December–March). Summer sea surface temperature data and vertical temperature profiles (Dodimead 1980, 1984) were used to calculate mean monthly water temperatures (May–October) at 100 m, where herring spend most of the day (Taylor et al. 1970). These estimates were used to calculate the mean temperature for all possible 2- through 6-mo segments during each season.

The effect of stock abundance was evaluated using a scaled biomass index for each stock which was calculated as the proportion of the 1974 adult biomass (Haist et al. 1986). These indices were derived for the summer feeding (May–October) and overwintering (December–March) aggregations inferred from tagging studies.

Multiple regression analyses were performed to evaluate the influence of temperature and stock biomass on herring reproductive characteristics for all subgroups combined. Mean sea temperatures and each of the two biomass indices were tested as independent variables. The maximum R^2 improvement technique of multiple regression analysis (SAS Institute Inc. 1985) was chosen to determine the best-fitting regression. Unlike forward, backward, and stepwise techniques, the maximum R^2 improvement procedure finds the best n-variable model by considering all independent variables. We used Sokal and Rohlf's (1981) F-test to evaluate the significance of improvement in R^2 resulting from adding new independent variables.

Results

Estimates of fecundity at the grand mean weight (126 g) for each subgroup are given in Table 2. Results of a two-way ANOVA indicated that fecundity differed significantly between years ($p = 0.0001$) but not locations ($p = 0.06$), though there is a tendency for mean fecundities to be higher in the Barkley Sound group. According to the results of a Tukey means comparison test, fecundity was 12.7% higher ($p < 0.05$) in 1983.

There were minor differences between the results obtained using weight- and length-specific fecundity that we attribute to interannual variations in the body condition factor. A two-way ANOVA of fecundity at the grand mean length (Table 3) indicated that year ($p = 0.0001$) but not location ($p = 0.06$) had a significant influence. However, Tukey's test showed that, even though the mean fecundity was highest in 1983, the year effect was due to the mean fecundities in 1983 and 1985

TABLE 3. Fecundity, total weight, and relative fecundity (eggs/g total weight) at the grand mean length (209 mm). Estimates of total weight were made from the common regression coefficient and subgroup-specific intercepts calculated from an ANCOVA of ln total weight − ln standard length regressions. Results of a GT-2 test indicated that the slopes of the weight–length regressions did not differ significantly ($p > 0.05$) among subgroups.

Parameter	1974	1980	1983	1984	1985
		Lambert Channel – Nanoose Bay			
Fecundity	22 123	27 584	32 334	26 977	28 356
Total weight	115	134	140	130	140
Relative fecundity	192	206	231	208	203
		Barkley Sound			
Fecundity	28 449	28 420	30 758	28 508	31 971
Total weight	131	144	133	133	146
Relative fecundity	217	197	231	214	219
		Kitasu Bay – Higgins Pass			
Fecundity	22 987	27 599	31 019	28 682	30 721
Total weight	120	143	133	130	141
Relative fecundity	192	193	233	221	218
		Kitkatla			
Fecundity	20 786	25 574	32 618	24 431	28 358
Total weight	125	141	141	133	134
Relative fecundity	166	181	231	184	212
		Port Simpson – Big Bay			
Fecundity	25 187	26 215	31 609	26 510	27 824
Total weight	128	139	143	137	143
Relative fecundity	197	189	221	194	195
		Juan Perez Sound – Skincuttle Inlet			
Fecundity	24 696	27 290	31 344	26 474	31 028
Total weight	127	143	147	128	150
Relative fecundity	194	191	213	207	207
		Inskip Channel			
Fecundity	28 062	27 515	30 677	27 542	29 554
Total weight	136	143	144	140	143
Relative fecundity	206	192	213	197	207

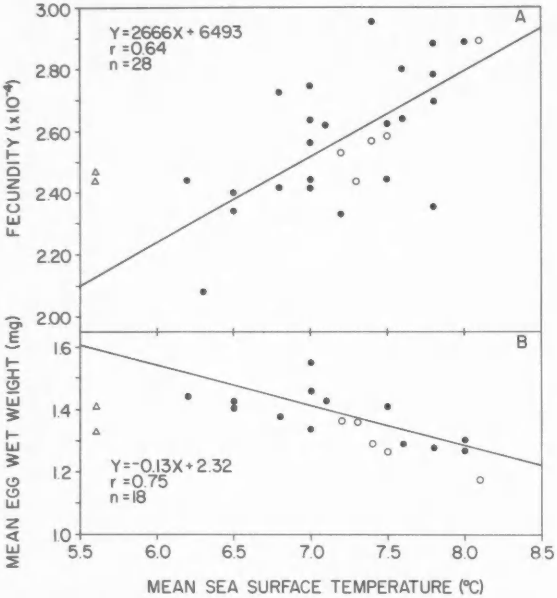

FIG. 2. Scatterplot and predictive regression equations for (A) fecundity and (B) mean egg weight at spawning all at the grand mean weight (126 g) against mean sea surface temperature 60−90 d before spawning. The open circles are for the Lambert Channel − Nanoose Bay spawners. The two triangles were outliers (see text) and therefore were excluded from all regression analyses. According to the predictive regression equation shown here, the fecundity Q_{10} = 2.9.

being significantly higher, and mean fecundity in 1974 being significantly lower, than all other years, respectively. Estimates of total weight at the grand mean length (Table 3), and trends in age-specific condition derived from size-at-age data (Tanasichuk, unpubl. data), show that British Columbia herring tended to be lighter at length in 1974. The two-way ANOVA was repeated using relative fecundity estimates for the grand mean length (Table 3). Results showed that year had a significant effect (p = 0.0001); Tukey's test indicated that this was because of high relative fecundities in 1983. Location also influenced relative fecundity significantly (p = 0.03); however, the effect was equivocal because Tukey's test could not identify where the estimates differed significantly (p = 0.05).

Ovary weight at spawning was estimated for the grand mean weight (Table 2). Results of a two-way ANOVA indicated that ripe ovary weight was not influenced significantly by year (p = 0.24) or location (p = 0.15).

Multiple regression analyses indicated that mean sea surface temperature 60−90 d before spawning best accounted for variations in fecundity at the grand mean weight (p = 0.0003, R^2 = 0.41) and length (p = 0.0054, R^2 = 0.26). Similarly, sea temperature over the same interval best accounted (p = 0.0003, R^2 = 0.57) for variations in egg weight. Summer sea temperatures, and summer and winter biomasses, had no significant effects ($p > 0.05$) on these characteristics.

The relationship between weight-specific fecundity and sea temperature 60−90 d before spawning is shown in Fig. 2A. Temperatures estimated for the Lambert Channel − Nanoose Bay group, which spawn in the Strait of Georgia, are probably the most accurate because their overwintering location is well-defined and the water is isothermal to 100 m. It seems likely that the actual correlation between water temperature and fecundity may be stronger than Fig. 2A suggests. If overwintering areas for northern herring were better defined and more accurate temperature estimates could be made for Barkley Sound herring, winter temperature might explain even more of the variance in fecundity. For this analysis, we determined that both points occurring at a temperature of 5.6°C deviated markedly from the trend (Fig. 2A; Studentized residuals >2 and leverage coefficients ≫0.13; Sokal and Rohlf 1981). Because we were uncertain where these fish overwintered, they were excluded from the final multiple regression analysis.

The weight-specific fecundity − winter sea temperature regression was used to scrutinize the location effect because of its equivocal statistical significance. Winter sea temperatures along the lower west coast of Vancouver Island, where the Barkley Sound spawners overwinter, tend to be warmer than elsewhere on the British Columbia coast. Consequently, the fecundity−temperature data pairs for these fish would be concentrated in the upper right quadrant of Fig. 2A. Two-way ANOVA's were repeated after excluding the data for the Barkley Sound fish. In all cases, the year effect persisted ($p <$ 0.0001) and the location effect was insignificant (p = 0.14, 0.19, and 0.15 for weight-specific fecundity, length-specific fecundity, and relative fecundity at the grand mean length, respectively).

The relationship between the mean egg weight at spawning (data from Table 2) and sea temperature 60−90 d before spawning is shown in Fig. 2B for each location and year subgroup where ripe ovary weight data were available (20 cases). There is a significant decrease in egg size at higher temperatures. As was the case in Fig. 2A, the points occurring at 5.6°C also deviated markedly from the trend and for the reason stated above were excluded from the analysis.

Our results indicate that while the size-specific ovary weight is remarkably stable between years, there is a significant trade-off between fecundity and egg size, which is significantly correlated with interannual variations in winter temperatures. Because fecundity and egg size are such strong components of Darwinian fitness, the trends shown in Fig. 2 have some important evolutionary implications. In the next section, we outline a theory of optimal egg size and define the conditions that must occur to make the observed trade-offs in fecundity and egg size adaptive.

Optimal Egg Size

Theory

To explore how changes in fecundity and egg weight affect fitness, we modified the early life history growth and survival model developed by Ware (1975). Briefly, the model recognizes that if there were no survival differences associated with egg size, then herring should produce as many small eggs as possible. This tendency, however, should be constrained by the fact that big eggs produce big larvae which tend to survive better because mortality in the sea is inversely related to body size (Peterson and Wroblewski 1984). This trade-off between egg numbers and size suggests that there may be an optimal egg size which maximizes survival during the early life history (see Ware 1975 for supporting details).

We used the following reasoning to estimate the optimum egg size − fecundity combination for Pacific herring. In British

Columbia stocks, mature fish average 126 g and produce a ripe egg mass (v) weighing 34 g. Thus the number of eggs spawned per female is given by v/W_0, where W_0 = egg weight (grams). After hatching, the instantaneous daily mortality rate (M) and growth rate (G) of the larvae vary inversely with their body weight (W) and directly with water temperatures (T):

$$(1)\quad \begin{aligned} M &= \frac{dN}{dtN} = AW^{-B}\,T^{C} \\ G &= \frac{dW}{dtW} = aW^{-b}\,T^{d}. \end{aligned}$$

Most biological processes tend to scale the same way with respect to body size, so we presumed b = B during the early life history because there is insufficient information on herring egg and larval mortality rates to enable us to calculate the mortality equation parameters. For this reason, we simplified the problem by defining Q as the ratio of the instantaneous daily mortality and growth rates:

$$(2)\quad Q = M/G = \frac{A}{a}\,T^{(C-d)}.$$

This enables us to evaluate the effect of arbitrary variations in Q on fitness. During the early life history stages, Q typically varies between 0.7 and 0.9 (Ware 1975).

Herring lay demersal eggs in a mass usually from 1 to 10 layers thick; to describe the mortality rate during this stage, we assumed that a herring egg has the same chance of dying each day during the incubation period as a larva of the same size. This is a key point because if the egg mortality is not size dependent, then there will be no optimum egg size. Instead there will be selection to make eggs as small as possible when $Q < 1$. There are good reasons, however, for believing that herring egg mortality varies inversely with egg size. Blaxter and Hunter (1982) noted that an increased thickness of spawn leads to lower survival because a low perfusion velocity of water moving past the egg mass is more likely to *limit* the respiration of the occluded eggs. Similarly, Alderdice and Hourston (1985) suggested that in naturally spawned egg masses, a lower packing density favors water movement and respiratory exchange, resulting in higher survival of occluded eggs. Taking the argument one step further, in nature, small eggs will have a higher packing density with smaller interstices between individuals, a poorer water movement and respiratory exchange, and hence a lower survival rate.

During the incubation period, herring eggs presumably die at an exponential rate. To obtain the number of eggs (per female) that survive to hatch (N_L), we can write

$$(3)\quad N_L = N_0 \exp\left(-IQaW_0^{-b}\right)$$

where Q is defined by eq. 2, I = incubation period (days), and $N_0 = v/W_0$ = initial number of eggs per average female. Experimental data published by Alderdice and Velsen (1971) indicate that the incubation time decreases with temperature according to the equation $I = 158T^{-1.06}$.

Once the larvae hatch, the time-dependent change in their body weight can be described by

$$(4)\quad W_t = (abt + W_L{}^{b})^{1/b}$$

where t = time (days) since hatching and W_L = weight (grams) of a newly hatched larva which we estimated as $0.39W_0$. Field measurements of the size of young herring from hatching to metamorphosis provide the following estimates for the growth parameters in eq. 4: $a = 0.033$ and $b = 0.19$. The number of larvae alive at any time (t) can be found by recalling that $M = QG$; hence:

$$\frac{dN}{dT} = -MN = -QaW^{-b}N$$

which can be integrated to give

$$(5)\quad N_t = \frac{N_L W_L{}^{Q}}{[abt + W_L{}^{b}]^{Q/b}}.$$

In accordance with current evolutionary thinking, we believe that the physiological process determining fecundity and egg weight in Pacific herring has a genetic basis and that the genotype which maximizes the number of surviving progeny has the highest fitness. To estimate fitness operationally, we determined the fecundity of an average 126-g fish, with a 34-g ovary mass, and then solved eq. 3, 4, and 5 for a fixed time t and for a number of different egg weights. This sequence of calculations was then repeated for selected incubation temperatures and arbitrary values of Q. For each sequence, the optimal egg size was defined as the one which left maximum number of survivors at time t.

Application

A preliminary analysis indicated that optimal egg size is sensitive to the duration of time (t) the selection process operates. In general, for any given value of Q the optimum shifts to smaller egg sizes as t decreases.

Thus, to begin, we must ask: What is the probable duration of time during the early life history over which maternal variations in egg size have evolved to maximize survival? If the observed interannual changes in egg size are adaptive, then, statistically, there must be a positive correlation between the temperature anomalies during a decisive period (apparently 60−90 d before spawning), when fecundity and egg weight are being determined, and temperature anomalies the following spring during the very early life history. This is a necessary condition so that when winter temperatures are warm, for example, and lead to a higher fecundity and reduction in egg size, in the long run there must be a selective advantage for producing small eggs and larvae the following spring. Figure 3 shows that the water temperature anomalies experienced by the Lambert Channel − Nanoose Bay group during their decisive period (December) are positively and significantly correlated with those in the following March from 1962 to 1985 ($r = 0.61$, $p < 0.01$). These results indicate that March temperature anomalies can be forecast quite accurately from December temperature anomalies, as both variables have the same sign 70% of the time. The monthly temperature anomalies for April and May are also significantly correlated (positively) with the December anomalies, and the June anomalies are just below the 5% level of significance (Fig. 4). Herring typically spawn in Lambert Channel around March 7 (Julian day 66), with the incubation period lasting, on average, about 2.5 wk until March 24 (day 83). Since the monthly temperature anomaly correlations persist until roughly the end of June (day 181), after which they break down completely, the data suggest that there is roughly a 90-d period after hatching where natural selection operates on interannual variations in herring egg size to maximize survival. In British Columbia herring stocks, this period covers events from spawning to metamorphosis (Fig. 4). In the model, we therefore assumed t = 90 d.

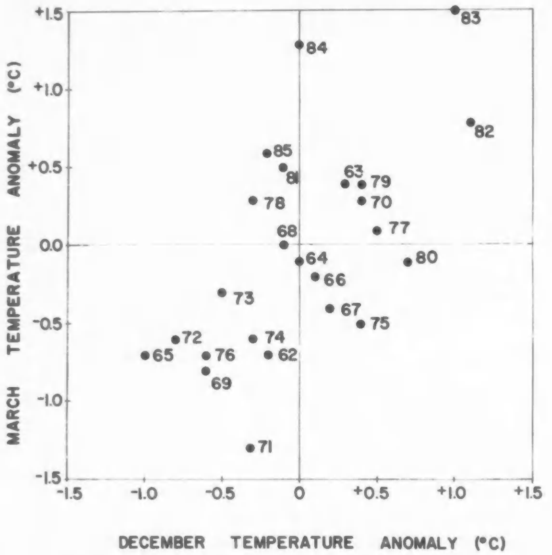

FIG. 3. Scatterplot of December temperature anomalies and subsequent March anomalies near Lambert Channel (Chrome Island Lighthouse), 1962–85. Anomalies were calculated by subtracting the grand mean monthly sea surface temperature from the respective mean monthly sea surface temperature. The figures indicate the year of spawning.

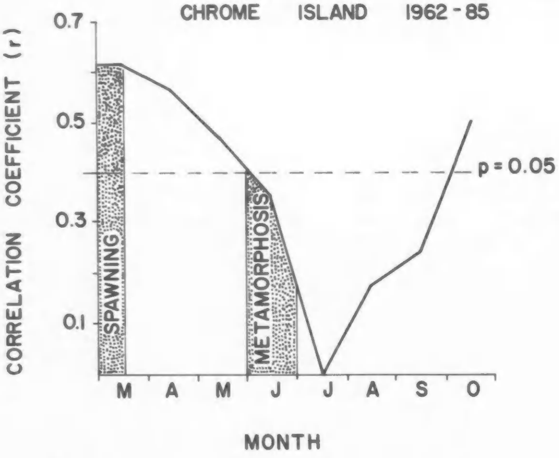

FIG. 4. Time-dependent trend in correlations between December temperature anomalies and subsequent monthly anomalies (March–October) at Chrome Island, 1962–85. The general timing of spawning and metamorphosis is indicated.

Simulation results indicated that the observed decrease in herring egg size with temperature will increase fitness when the growth rate Q_{10} is less than the mortality rate Q_{10}. Figure 5 shows the hypothetical fitness curves over 6–8°C, which covers the range in interannual temperature during the incubation period. These curves were obtained by estimating the value of Q required to fit the hypothetical, optimal egg size, at each temperature, to the observed size (Fig. 2B). In view of the continuity of the temperature trend, in the model, we assumed that the value of Q relevant to the temperature during the incubation period also applied to the 90-d period from hatching to metamorphosis. The following values of Q were obtained.

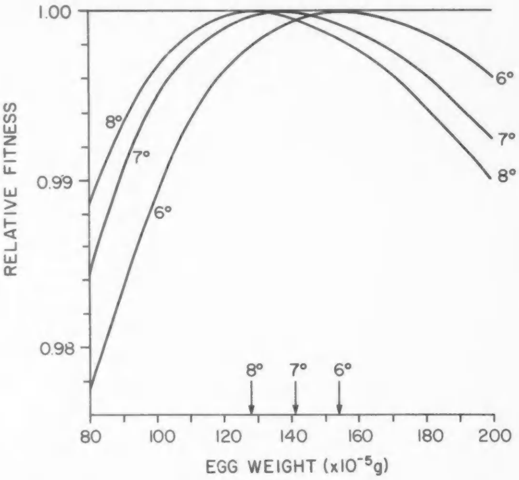

FIG. 5. Relative fitness of herring eggs of different sizes over the observed interannual range in water temperature during the incubation period. The curves were fitted to the model so the optimal egg size (relative fitness = 1) matched the observed mean egg size at each temperature. The arrows indicate the respective optima. The parameter values used to derive the curves are given in the text.

Temperature (°C)	Observed egg weight (g)	Q
6	0.00154	0.825
7	0.00141	0.870
8	0.00128	0.910

From this we calculated $Q = 0.448T^{0.341}$. It follows from our definition of Q that the value of the exponent in the foregoing equation is equal to $(C - d)$. Simulations indicated that the optimal egg weight will decrease with temperature as long as the exponent d in eq. 1 has a smaller value than exponent C, that is, as long as the growth rate Q_{10} is less than the mortality rate Q_{10}. When d exceeds C, the optimal egg size will *increase* with temperature.

Sensitivity analysis, for the case where the growth rate Q_{10} was less than the mortality rate Q_{10}, showed that optimal egg size is most responsive to changes in the growth rate and the Q ratio. All else being equal, to maximize fitness, egg weight should rise when either the growth rate or the Q ratio increases. In contrast, egg weight is expected to decline when the temperature rises and when the larvae require less time to reach metamorphosis.

Discussion

Size- and Age-specific Fecundity

The field results indicate that the size-specific fecundity of British Columbia herring is positively correlated with interannual variations in winter temperatures during a decisive period, roughly 60–90 d before spawning. A cursory examination of

J. Polder and J. Zijlstra's 1959 data (I.C.E.S. Unpubl. Doc. No. 84, Herring Committee) suggests, to us, that changes in water temperature might also be responsible for some of the between-year differences in length-specific fecundity of Dogger Bank herring. The equivocal effect of location in the present study supports our suggestion that temperature exerts a significant influence on size-specific fecundity, because British Columbia herring stocks overwinter in different locations and consequently may be exposed to different temperatures during the decisive period. The statistical significance of the intraannual (location) temperature effect was, in fact, a consequence of including the Barkley Sound spawners, which may overwinter in generally warmer water (Table 2). Although there appears to be some influence of location, the ANOVA's indicated that about 80% of the explained variation in size-specific fecundity and relative fecundity was accounted for by the year effect.

Our finding that the weight-specific fecundity of British Columbia herring is not influenced by variations in stock size clearly does not apply to all herring stocks. Anthony and Waring (1980) noted that the length-specific fecundity of Georges Bank herring decreased as stock abundance increased: the fecundity of a 30-cm herring declined from 107 000 to 67 000 (37%) when the number of age 4+ fish rose by 69%. Nikolskii (1969) described increases in length-specific fecundity for some herring populations which he attributed to reduced intraspecific competition for food because of low stock abundance. Unfortunately, neither report addresses the possibility that the observed changes in fecundity could simply reflect an increase in the body condition factor with weight-specific fecundity being constant. However, simulation studies of iteroparous, age-structured populations (Ware 1984) indicate that, when the adult mortality rate is low, and there is a survival cost associated with reproduction (Calow 1979), individual fitness may be enhanced if the weight-specific reproductive output (i.e. mature ovary weight) decreases in response to a rise in stock abundance. This happens because recently mature, and middle-aged adult, fish invest proportionally more of their energy resources in growth than in reproduction, and consequently experience a lower mortality rate (Ware 1984). Conversely, when the adult mortality rate is high, a constant size-specific reproductive output will tend to maximize fitness. In this context, it is significant that we found no evidence of density-dependent variations in weight-specific fecundity in British Columbia stocks of Pacific herring (which have relatively high natural mortality rates: $M \sim 0.45$). In contrast, density-dependent variations in size-specific fecundity have been reported in other stocks of Pacific and Atlantic herring which have much lower natural mortality rates: $M \sim 0.2$ (e.g. Georges Bank stock, Anthony and Waring 1980; Gulf of St. Lawrence and Scotian Shelf stocks, Messieh et al. 1985; southwest North Sea, Kamchatka and Hokkaido−Sakhalin stocks, Nikolskii 1969). Thus, the general trend in herring fecundity, with respect to stock abundance and the average adult mortality rate, is what one would expect on theoretical grounds if the individuals within these stocks were subject to a fitness-maximizing selection process.

Our results do not support Simpson's (1951, p. 18) hypothesis that "... the same balance of food supply to basic metabolic requirements which determines the growth in any year, may also determine at the critical period, several months or a year before each spawning, the actual numbers of eggs that will be developed for each spawning." Changes in stock biomass, and presumably food availability as a consequence, had no measurable effect on size-specific fecundity or ovary weight of British Columbia herring. Instead, fecundity seems to have been influenced primarily by a temperature effect operating during the winter, long after feeding had ceased.

It is important to note that although the size-specific fecundity of British Columbia herring is fairly constant over time (i.e. assuming no substantial variations in winter sea temperature), age-specific fecundity is not. It changes markedly in response to natural fluctuations in size-at-age. For example, the body weights of 3- to 5-yr-old herring off the lower west coast of Vancouver Island varied by a maximum of 30−46% between 1971 and 1980. Because fecundity is roughly proportional to body weight (see Table 1), it follows that age-specific fecundity must have fluctuated by the same amount. Presumably, differences in food availability, associated with interannual variations in stock biomass and oceanic conditions, are responsible for fluctuations in size-at-age and, hence, in fecundity-at-age.

Regulation of Fecundity and Egg Size

It is significant that the measured interannual variations in fecundity are not attributable to differences in ovary weight at spawning. Size-specific ovary weight remained remarkably constant over the years and between areas. Our results extend Ware's (1985) by showing that ovary weight of British Columbia herring does not change over wider ranges in temperature and stock abundance fluctuations than his observations were based on.

In British Columbia herring stocks, our results show that winter temperatures regulate fecundity, and because the mature ovary weight is constant, fecundity therefore determines egg size. We suspect that pre-ovulatory atresia (involution, or derecruitment, of young oocytes; Wallace and Selman 1981) provides the biological basis of this process which, in turn, is regulated by winter temperatures that modify gonadotropin levels during vitellogenesis. The occurrence of pre-ovulatory atresia in herring has been reported by Polder (1961), Bowers and Holliday (1961), Nikolskii (1969), and Hay (pers. comm.). It appears, as Hay (pers. comm.) and Blaxter and Holliday (1963) suggested, that the maximum potential fecundity is adjusted down under the influence of external factors, like temperature. Peter (1981) reported that fish gonadotropin may actually consist of a vitellogenic hormone and a maturation hormone. Cook and Peter (1980) found that the pituitary gland secretion rate of gonadotropin in maturing female goldfish (*Carassius auratus*) has a Q_{10} of 3.1, which is similar to the values we obtained for British Columbia herring stocks from the linear relationships between weight-specific ($Q_{10} = 2.9$), or length-specific ($Q_{10} = 3.0$), fecundity and sea temperature (Fig. 2A). Thus, in warm winters, gonadotropin levels in Pacific herring are probably higher 60−90 d before spawning, which appears to be a decisive period of vitellogenesis, and fewer eggs become atretic because more continue growing; the opposite response presumably happens in cold winters. This decisive period occurs at the stage (primarily IV of the Hjort maturity scale) of ovarian maturation that Bowers and Holliday (1961) and Hay (pers. comm.) have observed atretic pre-ovulatory oocytes in Atlantic and Pacific herring, respectively. Our belief that fecundity is determined some time prior to full maturation is supported by recent findings (Tanasichuk, unpubl. data) that it does not change significantly over the last month before spawning.

Herring Egg Size: Adaptive Significance

Without additional information it is impossible to say whether or not the observed trade-off in herring egg size and fecundity with temperature is adaptive. However, for the reasons discussed below, we believe that this trade-off has evolved to maximize the number of larvae that complete metamorphosis. There are two empirical measurements that can be made to test this hypothesis.

The first test considers the adaptive significance of the interannual variation in herring fecundity and egg size. According to our hypothesis, optimal egg size should decrease with temperature when the growth rate Q_{10} is less than the mortality rate Q_{10}. There is circumstantial evidence that this happens. Blaxter and Hempel (1963) found that the point of irreversible starvation in herring rises sharply with temperature ($Q_{10} \sim 2.7$), while predator rations are also expected to rise wth a Q_{10} between 2 and 3 to meet increasing metabolic demands. Hence, all else being equal, interannual variations in herring egg and larval mortality ought to increase with temperature by the same amount. With respect to growth, McGurk (1984) found that temperatures between 6 and 10°C did not affect the growth rate of experimental populations of Pacific herring. This evidence suggests that the growth rate Q_{10} of Pacific herring is significantly less than 2, while the mortality rate Q_{10} is probably higher, so the observed decrease in herring egg size, and rise in fecundity, with temperature should be adaptive. On the other hand, it is important to note that optimal egg size will *increase* with temperature when the growth rate Q_{10} is higher than the mortality rate Q_{10}. We note in passing that this possibility may be relevant to the early life histories of some other teleosts.

The second test of our hypothesis considers the within-season variability in herring egg size. First time, 2- and 3-yr-old recruit spawners lay their eggs about 2 wk later than the much larger and older repeat spawners (Hay 1985). The eggs of recruit spawners are about 20% smaller by weight. Stevenson (1962) found that late-spawned herring larvae grew about 0.4 mm/d, while the first-spawned cohort, or wave, grew more slowly at 0.3 mm/d. Presumably this difference is not due to the seasonal increase in temperature, but happens because late-spawned larvae hatch into a more advanced stage of the annual food production cycle. Using these growth rates, and the fact that late-spawned larvae reach metamorphosis in 67 d (instead of 90 d) after hatching, our model suggests that to increase their fitness, recruit spawners ought to produce larger eggs, *unless* their progeny experience a slightly lower Q ratio than earlier spawned cohorts. There is some circumstantial evidence supporting this possibility. The eggs laid 2 wk later by recruit spawners experience temperatures that are about 1°C warmer than the first-spawned cohort. Assuming a mortality Q_{10} of 2.5, this implies a 1.15-fold rise in the mortality rate. In contrast, the growth rate of the late-spawned larvae increases by a factor of (0.4/0.3) 1.33. Thus, as a rough approximation the Q ratio for late-spawned cohorts should be about 0.86 times less than the ratio for the early-spawned cohort. Above we noted that the average egg weight of repeat spawners (0.00141 g) will be optimal at 7°C when $Q = 0.87$. An independent run of the model utilizing the conditions applicable to the late-spawned cohorts indicates that the average egg weight of recruit spawners (0.00113 g) will be optimal at 8°C when $Q = 0.8$. This represents a 0.92-fold reduction in Q compared with the estimated 0.86-fold reduction. Thus, to explain why recruit spawners lay smaller eggs, the second test of our hypothesis is that the Q ratio for their offspring should be smaller (about 0.9 times less) than the ratio for the first-spawned cohort.

TABLE 4. Average reproductive and early life history characteristics of "spring"- and "autumn"-spawning races of Atlantic and Pacific herring.

Character	Spring	Autumn
1. Fecundity (27-cm female)	28 000[a]	64 000[a]
2. Dry egg weight (mg)	0.33[a]	0.17[a]
3. Temperature at spawning (°C)	7[b]	11[b]
4. Larval growth rate (mm/d)	0.32[c]	0.16[c]
5. Length at metamorphosis (mm)	35[d]	40[c]
6. Duration of larval phase (d)	90–120[c]	180–240[c]

[a] Schopka and Hempel (1973).
[b] Haegele and Schweigert (1985).
[c] Sinclair and Tremblay (1984).
[d] Stevenson (1962).

Our hypothesis can also explain seasonal differences in egg size, namely why "spring"-spawning herring lay bigger eggs than "autumn" spawners. Table 4 shows some of the relevant differences between these spawning groups. In general, the spring-spawning races produce eggs that are twice as big, and larvae that grow twice as fast, and reach metamorphosis in half the time of autumn spawners. It is also of interest to note that spring-spawned larvae appear to complete metamorphosis at a smaller size than their autumn-spawned counterparts. When we put the last four characteristics in Table 4 into our model, we find that in order to maximize the number of offspring that complete metamorphosis, spring-spawning females should produce an egg that is roughly 2.5 times larger and have a fecundity roughly 2.5 times lower than the same-sized autumn spawner. This is a completely independent test of our hypothesis, and the agreement with the observed differences in egg size and fecundity between the spawning groups is striking (Table 4). Part of the reason why our model predicts slightly larger differences is because it assumes that similar-sized females in both spawning groups have the same ripe ovary mass. This is an oversimplification, as Schopka and Hempel (1973) found that the total egg mass of herring of a given length is higher in autumn spawners than it is in spring spawners. We have not extended the model to consider the possible biological significance of differences in ovary weight. However, even in this simplified form, the model predicts, as Sinclair and Tremblay (1984) noted, that fecundity should be positively correlated with the duration of the larval stage in different spawning races. Our results support Sinclair and Tremblay's suggestion that greater accumulative mortality during a long larval phase will select for higher fecundity.

The foregoing discussion touches on the circumstantial evidence supporting the idea that the trade-off in herring egg size and fecundity has evolved to maximize the number of larvae that complete metamorphosis. If certain conditions are met, this unifying concept explains much of the variation in herring egg size at all scales: interannual, between season, and within season. Although the critical tests in each case have yet to be performed, we believe that the fecundity − egg size trade-off is adaptive and has been fine tuned by natural selection. In brief, we imagine a fitness-maximizing process where different genotypes in the population have different gonadotropin Q_{10}'s, or have different manifest effects of gonadotropin on pre-ovulatory atresia, so that similar-sized females end up with different fecundities and average egg sizes at spawning. Whatever the mechanism is, and it certainly may be more complicated than we suggest, it creates individual variation in egg size that is shifted by natural selection.

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NOTES

Ovarian Development and Oocyte Diameter as Maturation Criteria in Pacific Herring (*Clupea harengus pallasi*)

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Hay, D. E., D. N. Outram, B. A. McKeown, and M. Hurlburt. 1987. Ovarian development and oocyte diameter as maturation criteria in Pacific herring (*Clupea harengus pallasi*). Can. J. Fish. Aquat. Sci. 44: 1496-1502.

Seasonal changes in ovaries of Pacific herring (*Clupea harengus pallasi*) are described. Vitellogenic and resting oocytes could be distinguished histologically by size during early development. Resting oocytes had a mean diameter of about 80 μm and a maximum of about 150 μm. Oocytes larger than 150 μm were vitellogenic and indicate early sexual maturation. An alternative non-histological method of determining oocyte diameter, involving physical separation of oocytes, yielded good maturity estimates, rapidly and simply.

On décrit les variations saisonnières des ovaires du hareng du Pacifique (*Clupea harengus pallasi*). Au niveau histologique, les oocytes vitellogènes et inactifs peuvent être identifiés par leur taille initiale au cours du développement. Les oocytes inactifs ont un diamètre moyen d'environ 80 μm et un diamètre maximum d'environ 150 μm. Ceux dont la taille est supérieure à 150 μm sont vitellogènes et indiquent une maturation sexuelle précoce. Une autre méthode non histologique de détermination du diamètre des oocytes, comprenant leur séparation physique, permet d'obtenir rapidement et simplement de bonnes estimations de la maturité.

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Stock assessments of Pacific herring (*Clupea harengus pallasi*) in British Columbia are made during the spring spawning season and consider only sexually mature fish. Consequently, the estimate of spawning stock will be biased unless estimates are adjusted for changes in age at first maturity. It has been assumed that herring from southern British Columbia stocks were almost fully recruited (or sexually mature) during their third year although during some years substantial quantities of fish were recruited during their second year (Hourston 1980).

The migratory habits of herring complicate the testing of assumptions about recruitment to the adult biomass. Most Strait of Georgia herring migrate to waters offshore of the west coast of Vancouver Island (Fig. 1) during the summer months and return in the fall and winter (Taylor 1964). When captured and examined in the fall and winter, most herring of adult sizes are maturing sexually (Hay and Outram 1981; Hay 1985). However, it is possible that there could be substantial quantities of immature fish that overwinter offshore or reside in locations that are unknown. Therefore, assessment of the frequency of immature fish should be based on samples taken from offshore waters during the summer where aggregations of maturing and immature fish are mixed. At this time, however, ovaries and testes are small and sexual maturity is difficult to assess reliably by routine macroscopic examination such as the modified Hjort scale (Hay 1985). An alternative approach to maturity assessment involves measurement of oocyte diameters. Such a measurement permits a distinction between the immature or pre-vitellogenic oocyte and the developing or vitellogenic oocyte which have significantly larger diameters, even in small ovaries.

This note provides results of histological studies of oocyte development in herring. Also, an alternative to histological analyses based on physical separation of oocytes is tested and described.

Methods and Materials

Sample collection and preparation — Samples of adult herring were collected throughout the year from late July 1974 until the next June from a non-migratory, inshore population of herring residing near Porlier Pass and surrounding vicinity (Fig. 1). The fish were captured primarily with re-

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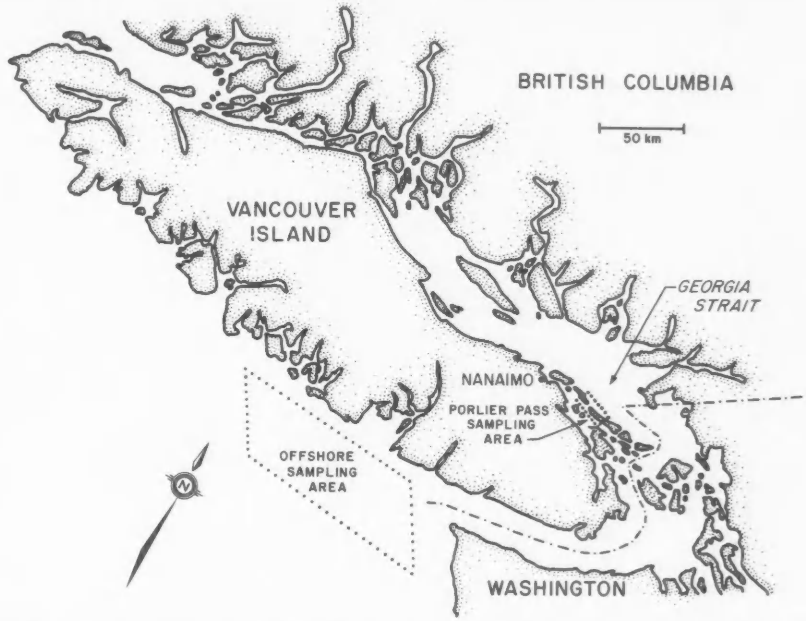


FIG. 1. Geographical origin of samples. Herring used for histological analyses were taken in the vicinity of Porlier Pass in the Strait of Georgia. Other herring samples were taken from banks off the west coast of Vancouver Island.

search trawls and commercial seine nets and frozen until subsequent laboratory analyses. Laboratory analyses at the Pacific Biological Station, Nanaimo, B.C., included estimation of standard length, total weight, sex, age, gonad weight, and modified Hjort maturity stage. For histological preparation, paired ovaries were fixed in Bouin's solution, imbedded in paraffin wax, and sectioned at 5 μm. To facilitate cutting of the more advanced stages with yolk-laden ova, the ovaries were soaked in a solution of 60% alcohol and glycerin prior to sectioning. The paraffin sections were stained either with Mallory's trichrom stain or hematoxylin and eosin.

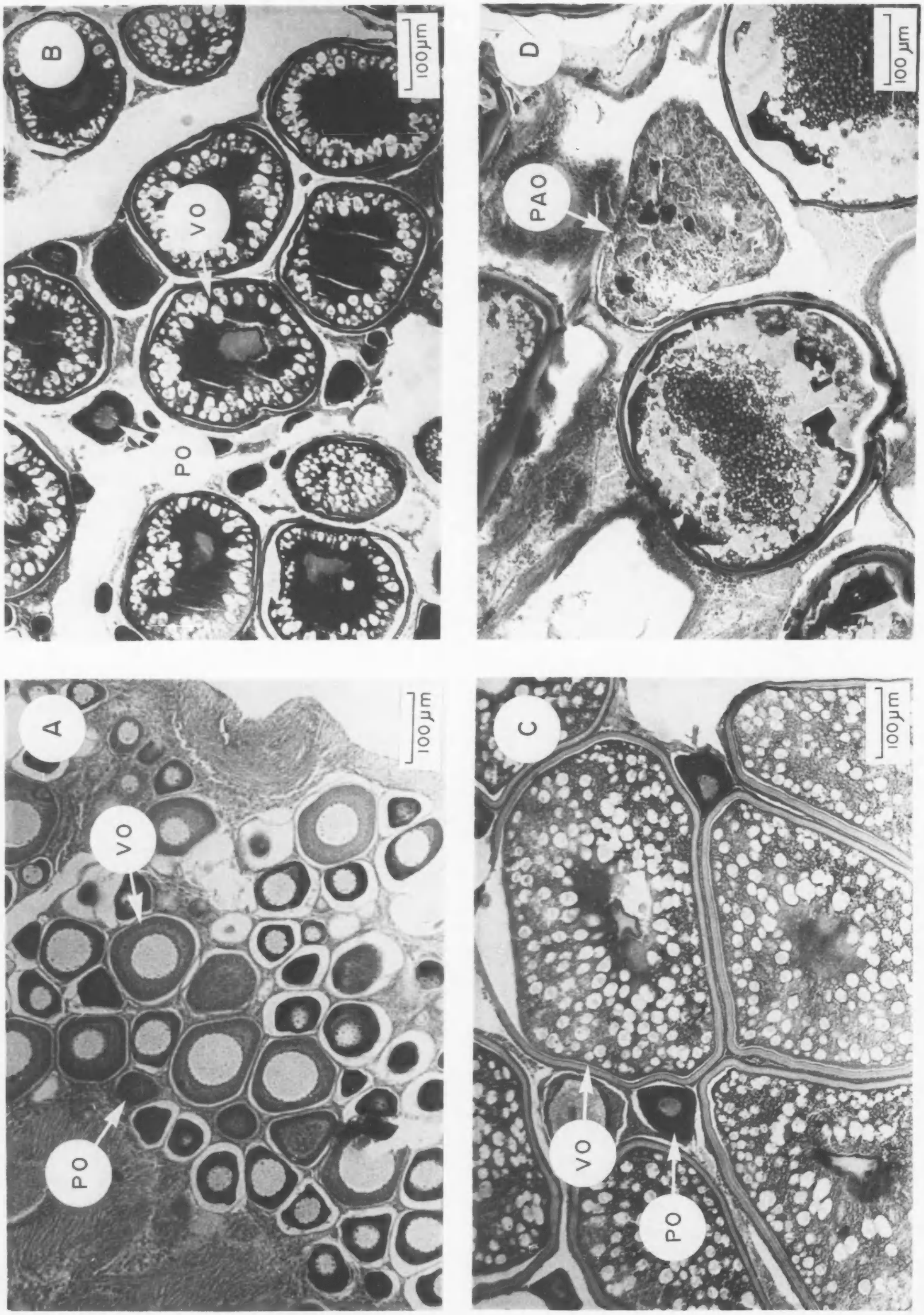
Different herring samples were collected by midwater trawl off the west coast of Vancouver Island (Fig. 1) during October and November 1980 and September 1981. Samples were frozen immediately after collection. They were thawed in the Nanaimo laboratory and fish were graded according to size and sex. Stratified subsamples were taken for each 20-mm length interval for a length range of 170−220 mm. A total of 82 females were examined: 26 from September 1980, 31 from November 1980, and 25 from September 1981.

The ovaries of each female were immersed in Bouin Hollande fixative for 24 h. Fixation longer than 1 d made the oocytes too brittle for sectioning, especially larger oocytes. Following fixation, the ovaries could be separated easily from surrounding tissue. Then the ovaries were embedded in paraplast, sectioned at 5 μm, and stained with hematoxylin and eosin. Fifty oocyte diameter measurements in 10-μm units were made from each ovary. Only oocytes that had been sectioned through the nucleus, close to the center of the oocyte, were measured, but otherwise, oocytes were selected randomly. This ensured that some of both the resting and developing, if present, oocytes were measured.

Non-histological examination of eggs — Ovarian tissue from several females captured in offshore locations in the winter was fixed in Bouin Hollande fixative for 10 d to harden the oocytes. This procedure would not be desirable for histology but the increased hardness allowed easy dispersion of the oocytes. Before removing the oocytes from the surrounding ovarian stroma, the ovary was placed in 70% ethanol to wash out the excess fixative and to avoid any excess swelling or shrinking. The ovary was then gently squeezed until it burst, releasing the oocytes. Aliquots of oocytes were transferred to a well slide with a pipette and oocyte diameters were measured under a microscope with an ocular micrometer. The only measurement possible was the maximum oocyte diameter. However, these measurements are readily obtainable and many measurements can be obtained in a short period. Very few eggs were damaged during the physical bursting of the ovary. Oocytes over a wide range of sizes could be measured in this fashion.

Results and Discussion

Histological changes during development — During routine biological analysis of British Columbia herring samples, maturation is estimated with reference to a modified eight-stage International Hjort maturity scale (Hay and Outram 1981; Hay 1985). Similar maturity scales are used for other herring work throughout the world (Naumov 1956; Polder 1961; Bowers and Holliday 1961; Iles 1964; Messieh 1975). For the purpose of comparison, the following description of histological changes at each maturity stage of Pacific herring follows similar descriptive formats used by Bowers and Holliday (1961) and Polder (1961) for Atlantic herring (*Clupea harengus harengus*). All data and measurements, however, are based


A
VO
PO
100 µm
B
VO
PO
100 µm
C
VO
PO
100 µm
D
PAO
100 µm

on microscopical examination of herring samples used in the present study.

Stage 1 ovaries are those of virgin fish. They are distinguished macroscopically by small thread-like ovaries and histologically by the immaturity of the developing primary germ cells and the absence of cellular yolk. Ovarian wall connective tissue of the ovary wall is thin, 5−25 μm.

In Stage 2 ovaries, some oocytes begin to acquire yolk vessicles (Fig. 2A). A chorion is differentiated and a connective tissue layer is formed around each oocyte containing the blood supply from the ovary. In early summer, early Stage 2 oocytes have diameters similar to pre-vitellogenic oocytes, while late Stage 2 vitellogenic oocytes (Fig. 2B), present mainly in late summer and early fall, were 300−370 μm in diameter with large nuclei diameters of 70−100 μm. Pre-vitellogenic resting oocytes, with mean and maximum diameters of about 80 and 150 μm, respectively, were present in all females. In an earlier similar analysis of Atlantic herring ovaries, Polder (1961) observed a maximum diameter of 190 μm. The ovary wall remained relatively thin, 7−40 μm, during this stage.

Stages 3−5 are primarily growth phases occurring from August to February (Fig. 2C) when the ovary almost fills the body cavity. Maximum oocyte diameters ranged from 670 to 870 μm through these stages. The ovary wall at these stages this time is about 90 μm thick. Atresia of some vitellogenic oocytes occurs at these stages (Fig. 2D).

Stage 6 occurs during March and April and is the last maturation phase: release of ripe eggs from their ruptured follicles into the lumen of the ovary. At the time of ovulation, oocytes take up water (Polder 1961; Gillis 1985) which causes a change in colour, transparency, and size of the ripe eggs. Oocytes are up to 1200 μm in diameter.

After spawning, spent ovaries are classified as Stages 7 and 8 of the Hjort scale or its modified version (Hay 1985). These stages are characterized, both internally and externally, by a state of disorganization: ruptured follicles, hemorrhages of the blood vessels, and folding of the ovary wall. Some unspawned eggs remained in some ovaries. From May until August, recovering ovaries passed through Stage 8 and underwent internal reorganization. Resting oocytes remained unchanged from Hjort Stage 2.

Macroscopically, it is difficult to distinguish between ovaries of recruit (virgin fish) and recovering spent fish (Stages 7 and 8). There are, however, several histological markers which could be useful. The folded and wrinkled ovary wall of the Stage 8 recovering ovary is about four times thicker (about 170 μm thick). Stage 8 ovaries may contain empty and collapsed follicles, unspawned ripe eggs undergoing degeneration, and unorganized connective tissue resulting from ovulation.

During maturation, two types of atresia or degeneration of the vitellogenic oocytes were observed. Pre-ovulatory atresia was found regularly in Stage 3−5 ovaries. Post-ovulatory atresia was observed in Stage 8 ovaries and involved a degeneration of ripe ova which remained in the ovary after spawning.

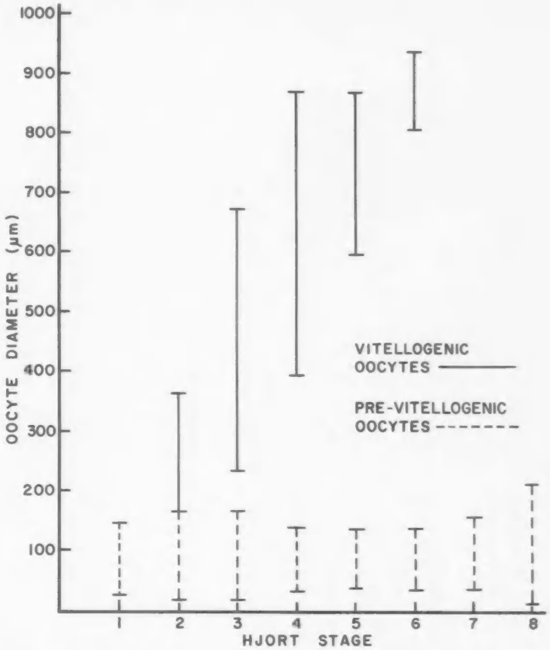


FIG. 3. Ranges of vitellogenic and pre-vitellogenic oocyte diameters by modified Hjort maturity stages.

In the course of one maturation cycle, ovaries contained both vitellogenic oocytes and the next year's resting oocytes. Therefore, if fecundity studies were carried out too early during the maturation cycle (around Stage 3), results could be misleading if resting and vitellogenic oocytes were not distinguished. Also, fecundity analyses conducted during Stages 3−5 might also be misleading because of some atresia of vitellogenic oocytes which occurs at these stages. Bowers and Holliday (1961) found that about 10% of ovaries of a few fish of Stages 4 and 5 had undergone follicular atresia and regression. Hay and Brett (1988) found a 20% decline in vitellogenic oocytes in captive herring held for 2 mo prior to normal spawning time.

Pre-vitellogenic and vitellogenic oocyte diameters at various Hjort stages are distinct except for Stage 2 (Fig 3). Analysis of the collection date and oocyte diameter (Hay 1985; Fig. 4) shows that the size frequencies of vitellogenic and pre-vitellogenic oocyte groups are distinct by late August or early September. Therefore, based on these analyses, we conclude that oocytes greater than about 150 μm must be vitellogenic and therefore are an indicator of early sexual maturation.

Maturity analysis in offshore samples — Oocyte diameters appear to be useful for identifying maturing herring collected in offshore waters. The frequency distributions of oocyte diameters, when pooled for each sample, are polymodal (Fig. 4).

FIG. 2. Microphotographs showing the distinction between pre-vitellogenic (PO) and vitellogenic (VO) oocytes and an example of pre-ovulatory atretic oocyte. (A) Early Stage 2: large oocytes are about 170 μm and have small yolk vessicles. The distinction between pre-vitellogenic and vitellogenic oocytes is not clear in many instances. (B) Late Stage 2: many yolk vessicles have accumulated and have reached the maximum diameter (30−35 μm) and migrated towards the oocyte center. The distinction between pre-vitellogenic and vitellogenic oocytes is unequivocal. (C) Stage 4: yolk granules fill occupy the cytoplasm surrounding the yolk vessicles. The vitellogenic oocytes are 400−500 μm in diameter. The pre-vitellogenic oocyte is about 120 μm in diameter. (D) Stage 4 showing a pre-ovulatory atretic oocyte (PAO).

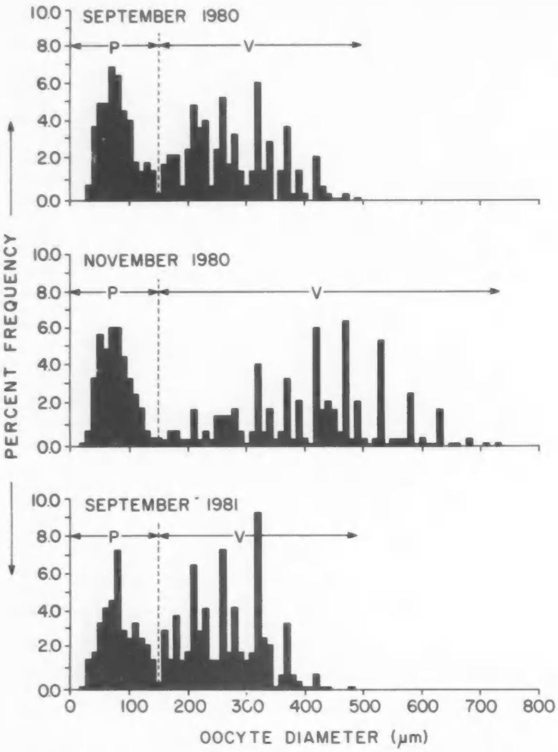


FIG. 4. Frequency distribution of oocyte diameters from herring collected off the west coast of Vancouver Island at three different times. The frequency mode from 0 to 150 µm corresponds to pre-vitellogenic oocytes (P) which is separated by a broken vertical line from the vitellogenic oocytes (V).

TABLE 1. Oocytes diameters (µm) from offshore samples.

Sample date	Pre-vitellogenic oocytes	Vitellogenic oocytes
September 1980		
Mean (±SE)	76.3(±19.3)	268.0(±13.0)
Minimum	58.0	165.0
Maximum	95.0	415.0
November 1980		
Mean (±SE)	74.5(±15.6)	390.7(±23.0)
Minimum	55.0	160.0
Maximum	88.1	585.6
September 1981		
Mean (±SE)	82.4(±20.4)	259.2(±10.3)
Minimum	61.8	176.0
Maximum	105.4	348.5

The smaller mode, corresponding to pre-vitellogenic oocytes, had mean diameters of about 75−80 µm (Table 1). The minimum and maximum means (for individual fish) of pre-vitellogenic oocytes varied from 55 to 105 µm (Table 1), although minimum and maximum oocytes measurements varied from 30 to 150 µm (Fig. 4). The larger modes (diameters >150 µm) represent the size distributions of the vitellogenic oocytes. The individual spikes occur in 10-µm increments corresponding to units of the ocular micrometer. In September 1980, two fish had only pre-vitellogenic oocytes and consequently were classified as immature. Similarly, four immatures were found in November 1980 and one in September 1981. Several of the fish classified as immature had one or two oocyte diameters which slightly exceeded the 150-µm criterion. Therefore, this criterion may be too strict; perhaps a few pre-vitellogenic oocytes exceed this diameter, but a more likely explanation is that pre-vitellogenic oocyte diameters greater than 150 µm reflect measurement error or oocyte distortion, especially when prepared from frozen tissue.

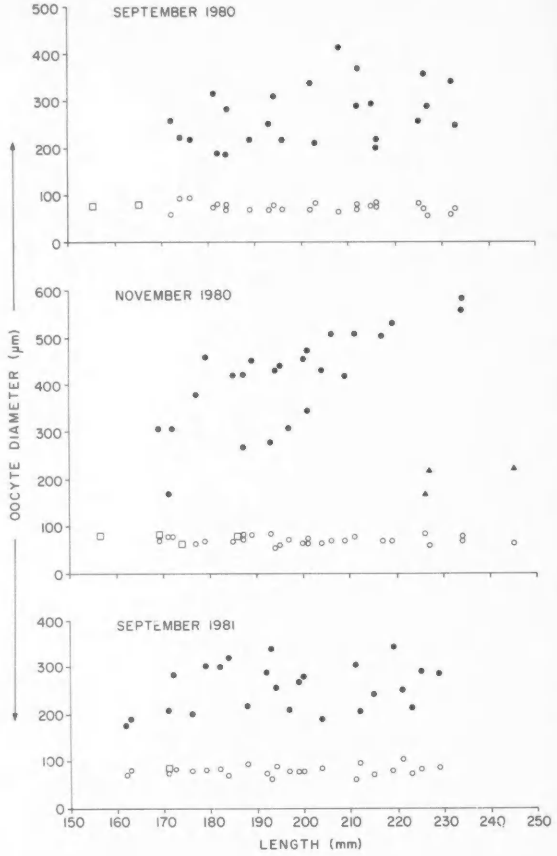


FIG. 5. Relationship between fish length and mean oocyte diameter in September 1980, November 1980, and September 1981. The open and solid circles represent pre-vitellogenic and vitellogenic oocytes, respectively. The open squares indicate immature fish with only vitellogenic oocytes. The solid triangles in November 1980 may represent fish with later maturation and spawning dates.

Vitellogenic oocyte diameters increased significantly with fish length and weight (Fig. 5). The correlation between fish length (or weight) and vitellogenic oocyte diameter was significant for the 1980 samples but not for 1981 (Table 2). There was no significant relationship between fish size and the pre-vitellogenic oocyte diameter at any time (Fig. 5). In November 1980, several larger females had relatively small oocytes. This could arise because single offshore midwater trawl catches

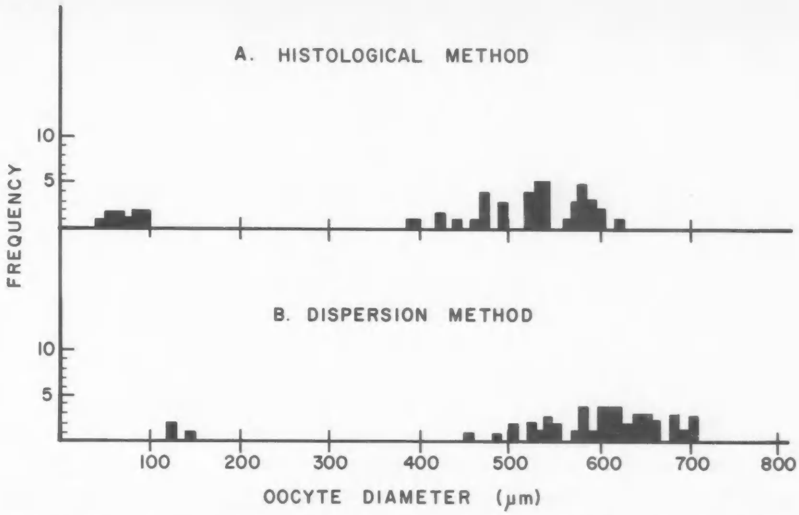


FIG. 6. Comparison of oocyte diameters from a single fish using (A) histological analyses of ovary sections and (B) an ooctye dispersion method.

TABLE 2. Correlation, r^2(%), and significance of oocyte diameter with lengths and weights of fish from offshore samples. Trial A in November 1980 included all data; trial B excluded three exceptional fish (distinguished in Fig. 4).

	Sample date	Pre-vitellogenic oocytes	Vitellogenic oocytes
Length	September 1980	8.3 (ns)	19.3 ($p < 0.05$)
	November 1980 (A)	0.1 (ns)	1.2 (ns)
	November 1980 (B)	0.1 (ns)	31.2 ($p < 0.01$)
	September 1981	0.7 (ns)	6.8 (ns)
Weight	September 1980	10.4 (ns)	21.9 ($p < 0.01$)
	November 1980 (A)	0.1 (ns)	3.6 (ns)
	November 1980 (B)	0.1 (ns)	38.1 ($p < 0.01$)
	September 1981	0.5 (ns)	12.0 (ns)

could consist of stock mixtures, each with different maturation schedules. Differences in maturation rates among individuals or stocks may reflect differences in spawning times, which range over 3 mo or more throughout southern British Columbia spawning locations. Earliest spawners are usually the largest, oldest fish (Hay 1985). Therefore, some variation in vitellogenic oocyte diameter would be expected in view of the variation in spawning times.

Comparison of histological and non-histological methods — Both pre-vitellogenic and larger vitellogenic oocytes were readily available from all parts of the ovary. The distribution of 50 oocyte diameters measured using the physical separation method was compared with that of oocyte diameters measured from histological slides (Fig. 6). The non-histological preparation had slightly smaller diameters, and relatively fewer oocytes in the pre-vitellogenic size class. In the histological preparations, slices may not necessarily pass through the middle of all oocyte; therefore, the diameter may be underestimated. The advantage of the dispersal method is the speed and simplicity. It is recommended for future work where only oocyte diameters are required.

Biological and management implications — A significant result of this work is the confirmation of atresia of vitellogenic oocytes in Pacific herring. Similar atresia was noted by Bowers and Holliday (1961) in Atlantic herring, although they discounted the significance of the phenomenon. We suggest that it may be vitally important in the determination of fecundity, or the number of vitellogenic eggs which ultimately reach ovulation by reducing the number of vitellogenic oocytes during maturation. This interpretation contradicts some earlier work (Naumov 1956; Bowers and Holliday 1961; Bagenal 1969) even though a similar interpretation of atresia has been suggested for other species (e.g. Scott 1962). For instance, Bowers and Holliday stated that "... fecundity in any one year is fixed at the immediate post-spawning age of the previous year, and that any external factors, eg., food supply, can have an influence then." An alternative explanation is that maximum possible fecundity, or the number of pre-vitellogenic oocytes, may indeed be fixed at the post-spawning time, but atresia of maturing vitellogenic oocytes may reduce the number of oocytes reaching full maturation. Such reduction may occur according to fish size and available energy for egg production.

The size frequency distributions of early oocytes provides an objective method to evaluate whether or not a herring is maturing. Information on age- and size-specific maturation, or maturity, is useful for management of Pacific herring where a substantial proportion of the total biomass may consist of newly recruited fish. This topic is discussed briefly by Hay (1985) and is under active investigation.

Acknowledgments

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Marking Nonfeeding Salmonid Fry with Dissolved Strontium

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Behrens Yamada, S., and T. J. Mulligan. 1987. Marking nonfeeding salmonid fry with dissolved strontium. Can J. Fish. Aquat. Sci. 44: 1502–1506.

Chemical marking is a simple, inexpensive, and rapid technique for marking young salmon. We have marked nonfeeding embryos by adding strontium to their rearing water. Strontium, a harmless element in its natural form, resembles calcium chemically and is therefore readily incorporated into bone. Our data show that the best time for inducing a Sr mark into nonfeeding salmon fry is after hatching, when bone development begins. The addition of 1 μg Sr/mL to the rearing water of salmon fry for a period of 49 d resulted in a 10-fold increase in their vertebral Sr concentration. As the salmon grew, the induced mark became diluted in a predictable manner, but was still detectable after 169 d.

Le marquage chimique est une méthode simple, rapide et économique pour l'identification de jeunes saumons. Les auteurs ont marqué des embryons qui ne se nourrissaient pas en ajoutant du strontium au bassin d'alevinage. Le strontium, un élément inoffensif dans sa forme naturelle, ressemble au calcium au niveau chimique; il est donc facilement incorporé dans la masse osseuse. Les données révèlent que le meilleur moment pour induire une marque au Sr chez un alevin qui ne se nourrit pas se situe après l'éclosion quand le développement des os commence. L'ajout de 1 μg/mL de Sr au bassin d'alevinage pendant 49 j a entraîné un découplement de la concentration de Sr dans les vertèbres. Au cours de la croissance, la marque induite s'est effacée selon les prévisions mais elle était encore identifiable après 169 j.

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Adult and juvenile fish have been marked in numerous ways; fin clips, freeze brands, tatoos, external tags, binary-coded stainless steel pins inserted into nose cartilage, or fluorescent marks in bones induced by adding tetracycline to the food. These techniques are difficult or impossible for small, nonfeeding fish. Because of this technical difficulty it has not been easy to follow the early growth and dispersal of pink (*Oncorhynchus gorbuscha*), chum (*O. beta*), and sockeye salmon (*O. nerka*). Unlike coho (*O. kisutch*) and chinook salmon (*O. tshawytscha*) and steelhead (*Salmo gairdneri*), these former three species are not raised to smolt size before leaving the hatchery or spawning channel, but enter the ocean or lake immediately after emergence from the gravel. Our study examines the feasibility of chemically marking hatchery salmon fry.

Chemical marking, a technique whereby a biologically rare element is substituted for one that is abundant in an organism's tissue, is not a new technique. Rubidium, which is chemically similar to potassium, has been used to mark Mexican bean beetles (Shephard and Waddill 1976); strontium, which is similar to calcium, has been used to mark goldfish (Ophel and Judd 1968) and salmon (Behrens Yamada et al. 1979; Behrens Yamada and Mulligan 1982). In all these studies the chemical mark was induced through the diet. Preliminary experiments (S. Behrens Yamada and T. J. Mulligan, unpubl. data) indicated that salmon also take up elements from the ambient water. This study examines the feasibility of chemically marking nonfeeding salmon fry by adding stable Sr to the water supply.

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Materials and Methods

Experiments were run during two successive salmon breeding seasons. In the first experiment a low concentration of Sr was added to the water throughout the incubation and fry stages to determine the time during which Sr uptake is most rapid. In the second experiment, a more easily detected mark was induced by adding a higher concentration of Sr for the fry period.

Experiment 1 — Sockeye eggs and sperm from Fulton River in northern British Columbia were collected on September 29, 1977, and taken to Rosewall Creek Hatchery on Vancouver Island. At the hatchery, eggs were fertilized, water hardened, and distributed equally among 12 incubating trays. (All water exposure, including the water hardening, was at the hatchery in water with known Sr content.) The trays were placed in two stacks: one treatment and one control stack. A nominal concentration of 0.1 μg Sr/mL was obtained for the treatment stack by adding a stock solution of $SrCl_2 \cdot 6H_2O$ to the water supply using a peristaltic metering pump. Salmon eggs/fry stayed in the incubation trays for 110 d, after which they were transferred to tanks and fed starter mash.

The developing salmon were sampled from fertilization (day 0) to feeding fry at day 153. Samples, consisting of whole eggs or whole fry, were prepared by freeze-drying for 2 d and grinding them for 3 min in a mixer mill. A 0.1-g quantity of each powdered sample was pressed into a pill 1.27 cm in diameter using a die and hydraulic press with an applied load of 1700 kg/cm^2. The sample pills were analysed for their Ca and Sr concentrations using X-ray fluorescence spectroscopy. Quantitative determination of Ca and Sr in each sample was accomplished using the method described by Giauque et al. (1973). A full description of the equipment and methods can be found in Mulligan et al. (1983).

Experiment 2 — Eggs and sperm from 36 female and 7 male coho salmon from Big Qualicum Creek Hatchery, located on Vancouver Island, British Columbia, were collected December 12, 1978, and taken to Rosewall Creek Hatchery. There eggs were fertilized, water hardened, and divided equally into 36 incubation trays. These trays were arranged into six stacks such that each stack would hold two empty trays on top to allow for adequate mixing of the Sr stock solution and six egg-filled trays below. A nominal concentration of 1.0 μg Sr/mL (10 times higher than in the previous experiment) was obtained for three of the stacks from day 0 or January 24 (12 d prior to hatching) to day 49 or March 14, 1979, at which time fish were taken out of trays and put into four circular tanks. (Note that day 0 in this experiment is defined as the day on which treatment of the water supply began.) Two tanks contained Sr-treated fish and two contained control fish. This move to tanks from incubation trays ended the treatment period for the Sr-treated fish.

One tank of fish for each treatment received standard Oregon Moist Pellets containing from 100–160 μg Sr/g throughout the remainder of the experiment, while the other tank of each treatment received a specially formulated diet from day 59 to day 115. It was hoped that this diet, devoid of bone meal, would be low in Sr and thus not interfere with the induced Sr mark. Subsequent analysis of this diet revealed a Sr concentration of 50 μg/g.

Sampling of salmon started after hatching on day 33 (February 26) and continued until day 218 (August 30, 1979). Fry were measured and weighed while damp. Backbones were dissected, cleaned on a paper towel, allowed to air dry for 24 h, and weighed. Single backbones were placed into 2 or 3 mL of leaching solution consisting of 0.1 *M* HCl in 10% glycerol (Ophel and Judd 1968). After 3 d the leaching solution was directly aspirated into a flame photometer. The relative concentrations obtained by this rapid leaching, atomic absorption spectroscopic technique compare well with those obtained by acid digestion (Ophel and Judd 1968) and by the more time-consuming X-ray fluorescent spectroscopy (Behrens Yamada and Mulligan 1982).

Results and Discussion

Experiment 1 — The Ca and Sr uptake by developing salmon form parallel patterns which can be broken down into three distinct periods (Fig. 1): (i) incubating eggs — from fertilization to hatching (day 0–83); (ii) fry — from hatching to "swim up" (day 83–111); (iii) feeding fry — after "swim up" (day 111). No change in the Ca and Sr concentrations was observed in the egg stage (Fig. 1). The concentration of Ca remained around 3000 μg/g that of Sr around 17 μg/g. Similar results were observed for the same period of development in the Ca concentrations of Atlantic salmon (*Salmo solar*) and rainbow trout (*S. gairdneri*) (Hayes et al. 1946; Ogino and Yasuda 1962; Zeitoun et al. 1976). This period of cell division, gastrulation, and organogenesis depends mainly on nutrients and minerals stored within the yolk and perivitelline fluid and not as much on the minerals in the ambient water. Any attempt to induce a chemical mark during this period appears to be futile.

Fry still depend on the yolk for nourishment; however, when the egg membrane breaks, they are able to take up ions from the ambient water through their gill and intestinal epithelia and through the highly vascularized surfaces of the yolk. In 28 d the Ca concentration of fry steadily increased from 3300 to 6900 μg/g. During the same time, the Sr concentration in control fry increased slightly from 15 to 19 μg/g, while that of the Sr-treated fry doubled from 16 to 32 μg/g (Fig. 1). Similar increases in the Ca concentration after hatching were observed for Atlantic salmon and rainbow trout (Hayes et al. 1946; Ogino and Yasuda 1962; Zeitoun et al. 1976). Two of these studies attribute most of this increase in Ca to uptake from the ambient water. Hayes et al. (1946) also observed an increase in P which paralleled that of Ca. These increases in the major components of bone during this active phase of development are correlated with rapid bone development. Thus the desired time to start inducing a Sr mark appears to be after hatching.

Feeding fry continue to concentrate Ca and Sr in their tissues. Now they have two sources for Ca and Sr uptake: the

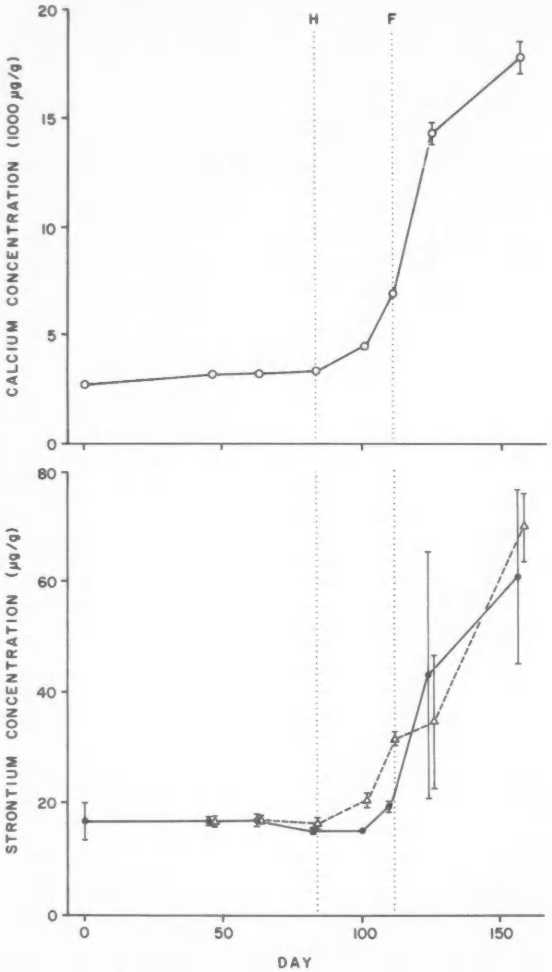

FIG. 1. Calcium and Sr concentrations in whole-fish samples of developing sockeye salmon. H indicates time of hatching; F indicates initiation of feeding. Open circles represent Ca concentrations in both the control and Sr treatments; solid circles represent Sr concentrations in the control; open triangles represent the Sr concentrations in the Sr treatment; bars indicate the 95% confidence limits of the mean. Confidence limits smaller than the symbols are not indicated.

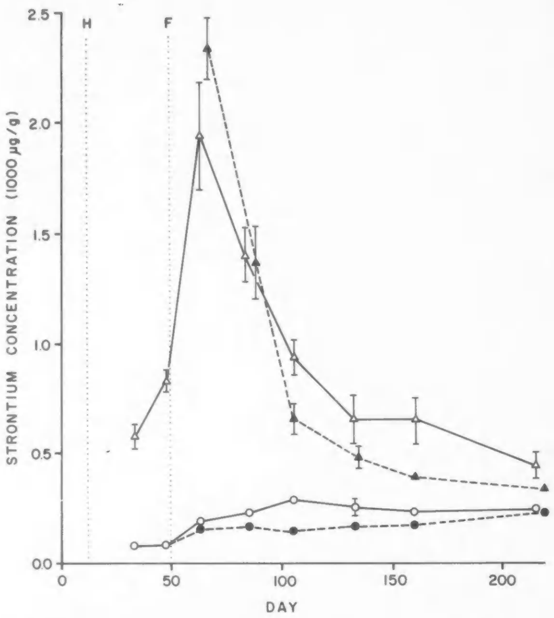

FIG. 2. Strontium concentrations in backbone samples of young coho salmon. H indicates time of hatching; F indicates initiation of feeding. Triangles represent the Sr concentration in the Sr treatments; circles represent the Sr concentrations in the control treatment; solid circles and triangles represent the Sr concentration in the fish which received the low-Sr diet from day 59 to day 115; bars indicate the 95% confidence limits of the mean. Confidence limits smaller than the symbols are not indicated.

ambient water and the food. The Ca concentration of Rosewall Creek water was 10.8 µg/mL with undetectible levels of Sr whereas the Ca concentration in fish food was 2500 µg/g with a Sr concentration of 150 µg/g. Hence, feeding fry probably take up Ca from both sources, while Sr is absorbed mainly from the food. The weak Sr mark induced during the fry stage quickly became masked after the fish began feeding. The great variability observed in the Sr concentrations of feeding fry may reflect individual variation in feeding rates and thus Sr uptake.

Experiment 2 — A more easily observed Sr mark can be induced into salmon vertebrae by increasing the added Sr in the rearing water of nonfeeding fry to reach a concentration of 1 µg/mL (Fig. 2). Forty-nine days of Sr treatment resulted in a 10-fold increase in bone Sr from 79 µg/g for control fry to 834 µg/g for treated fry. The maximum Sr concentration of 1900 and 2300 µg/g did not coincide with a cessation of treatment, but occurred 12 d later. This lag in response may represent the time for soft tissue Sr to reach an equilibrium with bone Sr. After initiation of feeding, the Sr concentration of the control fish receiving the standard diet increased to around 250 µg/g by day 85 and then leveled off; that of the control fish receiving the low-Sr diet from day 59 to day 115 leveled off around 160 µg/g while receiving the special diet and then increased gradually to 230 µg/g by day 218 (Fig. 2).

After reaching a peak concentration of ~200 µg/g at day 63, the Sr concentration in the vertebrae of Sr-treated fish decreased exponentially to 450 and 340 µg/g by day 218 (Fig. 2; Table 1). As the fish grew, the Sr mark in the treated fish represented a progressively smaller proportion of the total Sr content. The more rapid decrease in the low-Sr-food tank is correlated with a faster growth rate on this special diet. The calculations in Table 1 demonstrate that the reduction in the induced Sr mark is not due to loss of the mark but due to dilution of the mark by growth. The difference in Sr concentration between treated and control fish decreased between day 63 and day 218; however, the amount of induced Sr per vertebra remained constant at ~2.9 µg for standard diet tanks and ~3.4 µg for low-Sr-diet tanks.

Dilution of the Sr mark with growth was also demonstrated in a previous study in which the Sr mark was induced orally (Behrens Yamada et al. 1979; Behrens Yamada and Mulligan 1982). These authors found that the amount of Sr in the

TABLE 1. Vertebral Sr content for Sr treatment and control fish. Numbers in parentheses represent 1 SD.

	Sr treatment			Control			
Day	Mean weight of vertebra (g)[a]	Mean Sr concentration (μg/g)	Total Sr per vertebra (μg)	Mean weight of vertebra (g)[a]	Mean Sr concentration (μg/g)	Total Sr per vertebra (μg)	Difference in total Sr per vertebra (μg)
				Standard diet			
63	0.0013 (0.0004)	1945 (123)	2.53 (0.79)	0.0013 (0.0004)	192 (7)	0.25 (0.08)	2.28 (0.80)
85	0.0025 (0.0006)	1406 (65)	3.51 (0.86)	0.0025 (0.0006)	232 (8)	0.58 (0.14)	2.93 (0.87)
105	0.0043 (0.0010)	935 (42)	4.02 (0.95)	0.0043 (0.0010)	285 (9)	1.23 (0.29)	2.80 (0.99)
133	0.0075 (0.0011)	659 (57)	4.94 (0.84)	0.0060 (0.0011)	254 (24)	1.52 (0.31)	3.42 (0.90)
160	0.0093 (0.0020)	658 (59)	6.12 (1.43)	0.0095 (0.0020)	244 (8)	2.32 (0.49)	3.80 (1.51)
218	0.0220 (0.0067)	449 (31)	9.88 (3.08)	0.0260 (0.0068)	250 (5)	6.50 (1.70)	3.38 (3.52)
				Weighted mean of difference = 2.9 (0.5)			
				Low-Sr diet			
63	0.0013 (0.0004)	2344 (73)	3.05 (0.94)	0.0013 (0.0004)	157 (7)	0.20 (0.06)	2.84 (0.94)
85	0.0031 (0.0006)	1370 (84)	4.25 (0.86)	0.0030 (0.0006)	172 (9)	0.52 (0.11)	3.73 (0.87)
105	0.0070 (0.0011)	666 (34)	4.66 (0.77)	0.0062 (0.0011)	149 (8)	0.92 (0.17)	3.74 (0.79)
133	0.0093 (0.0020)	485 (28)	4.51 (1.00)	0.0103 (0.0020)	174 (11)	1.79 (0.37)	2.72 (1.07)
160	0.0147 (0.0020)	397 (14)	5.84 (0.82)	0.0145 (0.0020)	177 (7)	2.57 (0.37)	3.27 (0.90)
218	0.0280 (0.0069)	339 (13)	9.49 (2.37)	0.0220 (0.0067)	228 (4)	5.02 (1.53)	4.48 (2.82)
				Weighted mean of difference = 3.4 (0.5)			

[a] The vertebra weights were estimated from a regression of vertebra weight on fish weight using a subsample of the fish from this experiment.

backbone of control and treated fish can be expressed for any time after treatment as, respectively,

$$(1)\quad Sr_2W_2 = Sr_1W_1 + K(W_2 - W_1)$$

$$(2)\quad Sr'_2W'_2 = Sr'_1W'_1 + K(W'_2 - W'_1)$$

where the subscript 1 represents the time of initial Sr determination and 2 represents a later determination, the primed quantities represent Sr-treated fish and the unprimed quantitites represent controls, Sr is the Sr concentration, W is the weight of the fish (proporitonal to the weight of the backbone), and K is a value representing the amount of Sr taken up from the environment, between time 1 and 2, per unit of body weight gained.

Equation (1) then states that the total amount of Sr in the backbone of control fish at time $t = 2$ can be expressed as the product of the weight times the Sr concentration and is equal to the total amount of Sr at time 1 plus some additional amount which is proportional to the difference in weights at time 1 and 2.

Equation (2), which describes the Sr-treated fish, is identical in form to equation (1). Notice that K is the same in both equations, since we assume that both groups of fish accumulate Sr from the environment at the same rate. If we assume that growth of the two groups of fish is proportional,

$$\frac{W_1}{W_2} = \frac{W'_1}{W'_2},$$

then equations (1) and (2) can be solved to give

$$(3)\quad \frac{Sr'_2 - Sr_2}{Sr'_1 - Sr_1} = \frac{W_1}{W_2}.$$

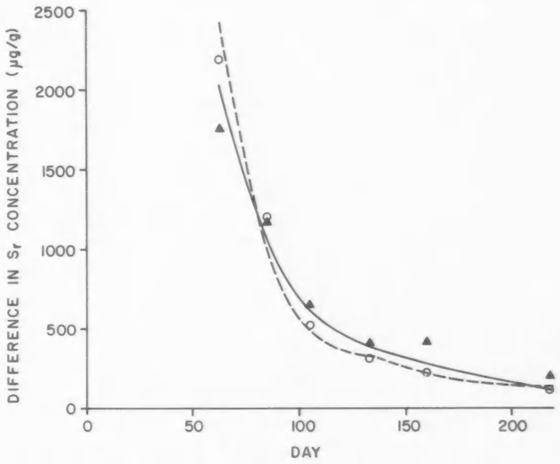

FIG. 3. Difference in Sr concentration between the treated and control fish versus time. Triangles represent fish fed the standard diet; the solid line is the fit to this data using the formulae in the text; circles represent the low-Sr diet fish; the broken line represents the corresponding fit.

The difference in concentration between treated and untreated fish is inversely proportional to weight.

We can use equation (3) to calculate the expected difference in Sr concentration between the treated and control fish (Fig. 3). The difference in growth rate of our fish while fed the low-Sr diet is responsible for the unusual looking "bump" in the curve of the figure for this group of fish. While we have no information on the range of temperature, Sr concentrations, etc., over which these equations will apply, they seem to provide a reasonably accurate description of our measurements.

Application of chemical marking technique — We have successfully marked incubating salmon by adding stable Sr to their rearing water. As in a previous laboratory study with goldfish (Ophel and Judd 1968) and a field study with coho salmon (Behrens Yamada et al. 1979) Sr did not effect the fish in any deleterious way, but simply substituted for Ca in the calcium phosphate bone matrix. We have found that the best time for inducing a Sr mark into incubating salmon is after hatching, when bone development begins.

Our findings can be applied to mark nonfeeding fry. At present, no simple marking technique exists for following the growth and dispersal of pink, chum, and sockeye salmon fry. Unlike coho, chinook, and steelhead, these three former species are not raised to smolt size before leaving the rearing facility, but enter the ocean or lake soon after incubation. By adding Sr to the water, a chemical mark can be induced without handling the fish.

This technique could also be used to evaluate the effect of hatchery boxes on salmonid production. Recently, volunteer groups have been trying to increase the escapement of steelhead, trout and coho and chinook salmon by installing hatchery boxes in streams. It is not known whether the fry produced in hatchery boxes have a beneficial effect by increasing the run or a detrimental effect by competing with wild fry for rearing habitat. By marking fry inside the hatchery boxes, one could compare the survival and growth rate of Sr-marked hatchery box fry with that of unmarked wild fry.

The Sr marking technique can be applied directly to species that enter freshwater. A concentration of 1 μg Sr/mL applied for 49 d during the fry stage resulted in a detectible Sr mark 169 d later. The fish at that time had reached a weight of 5 g. If fish in nature grow less rapidly than in a hatchery, the mark would last longer. If a shorter marking time or a stronger mark is desired, one could try increasing the Sr concentration.

If fish are to be followed for an extensive time in estuaries or oceans, the marking technique would have to be modified. Since Sr is a major constituent of seawater (8 μg/mL), any induced Sr mark would soon be masked. Results from a previous field experiment, in which coho smolts were fed an additional 10 mg Sr/g in their diet for 60 d, suggest that an induced mark (30 × control fish concentration) was still detectible after 5 mo of ocean residence (Behrens Yamada et al. 1979). Depending on growth rates and Sr concentration of the ambient water and food, one might expect an induced mark of a 10-fold increase in Sr to last about 1–2 mo. This Sr mark could be intensified by increasing the Sr concentration of the water or by retaining the fish a couple of weeks after swim up and feeding them a Sr-enriched diet. The feasibility of using other benign elements such as manganese, rubidium, or cesium, which are rarer than Sr in seawater, could also be investigated. Since vertebrae and scales grow concentrically, it is possible to remove that portion of a fish's vertebra or scale which was added during seawater growth. The remaining central portions, representing the freshwater growth, will still have the same Sr concentration as observed before release (Behrens Yamada and Mulligan 1982).

We hope that this study will encourage management and research applications of our findings. The ability to mark nonfeeding salmon fry by a method which does not require handling of individual fish and which is relatively long lasting should be useful for many problems.

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Lack of a Depth Effect on the Growth of the Air-Breathing Catfish, *Clarias macrocephalus*

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In juvenile *Clarias macrocephalus*, distance travelled to breathe air increased strongly with depth, but growth rate was not significantly affected. Forty-seven fish (4.60 ± 0.12 g, 8.4 ± 0.07 cm, mean ± SE) were initially raised for 28 d in individual cages (30, 60, 90, and 118 cm deep) at 27°C and 0.72 mg $O_2 \cdot L^{-1}$, with a daily ration of 6% body weight. Distance travelled increased from 15.5 $m \cdot h^{-1}$ at 30 cm to 51.2 $m \cdot h^{-1}$ at 118 cm. When fish were subsequently maintained without food for a further 14 d, at 0.15 mg $O_2 \cdot L^{-1}$, distance travelled averaged 6.6 and 22.7 $m \cdot h^{-1}$ at 30 and 118 cm, respectively. Despite the increase in activity, depth had no significant effect on specific growth rate of either fed (3.62 ± 0.08$\% \cdot d^{-1}$) or fasting (−0.70 ± 0.03$\% \cdot d^{-1}$) fish. Strong effects of depth on the growth of air-breathing fishes may not be as general as previously suggested.

La distance parcourue par *Clarias macrocephalus* juvénile pour respirer de l'air augmente nettement avec la profondeur quoique le taux de croissance ne soit pas touché de façon significative. On a élevé 47 poissons (4,60 ± 0,12 g, 8,4 ± 0,07 cm, moyenne ± ErT) pendant 28 j dans des cages individuelles (30, 60, 90 et 118 cm de profondeur) à 27°C et 0,72 $mg \cdot L^{-1}$ de O_2 avec une ration quotidienne équivalente à 6 % du poids corporel. La distance parcourue est passée de 15,5 $m \cdot h^{-1}$ dans les cages de 30 cm de profondeur à 51,2 $m \cdot h^{-1}$ dans les cages de 118 cm de profondeur. Gardés pendant 14 autres jours sans nourriture à 0,15 $mg \cdot L^{-1}$ de O_2, les poissons ont parcouru une distance moyenne respective de 6,6 et 22,7 $m \cdot h^{-1}$ dans les cages de 30 et 118 cm de profondeur. Malgré l'augmentation de l'activité, la profondeur n'avait aucune incidence significative sur le taux de croissance spécifique des poissons nourris (3,62 ± 0,08 $\% \cdot j^{-1}$) ou à jeun (−0,70 ± 0,03 $\% \cdot j^{-1}$). L'influence marquée de la profondeur sur la croissance des poissons à respiration aérienne n'est peut-être pas aussi générale qu'on le croyait.

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For aquatic organisms that use air breathing or aquatic surface respiration to obtain oxygen, travel to and from the surface may be an important component of the cost of respiration (Kramer 1987). Behavioural evidence for this cost is provided by a number of amphibians and fish which reduce their surfacing frequency when living at greater depths (Bevan and Kramer 1986). In the benthic catfish *Clarias macrocephalus*, the reduction in air-breathing frequency is associated with increased water-breathing frequency; when dissolved oxygen levels are near saturation, the reduction in surfacing counterbalances the increase in depth, so that distance travelled (metres per hour) remains constant as depth increases from 60 to 230 cm (Bevan and Kramer 1987). However, under hypoxic conditions such complete compensation is not possible, and several studies suggest that the temporal and energetic costs of surfacing can be large, especially when the surfacing distance or frequency is great. For example, *Channa striatus* (4.5 cm total length, TL) spent over 4.5 $h \cdot d^{-1}$ on surfacing activity when reared at 40 cm (Pandian and Vivekanandan 1976). In two genera of air-breathing fish, *Channa* (Pandian and Vivekanandan 1976; Vivekanandan and Pandian 1977; Vivekanandan 1977b) and *Heteropneustes* (Arunachalam et al. 1976), and two species of tadpoles, *Rana pipiens* (Feder and Moran 1985) and *R. tigrina* (Pandian and Marian 1985), a reduction in either feed conversion efficiency or growth rate with increasing depth has been observed. In addition, a preliminary study of *C. macrocephalus* also suggested a reduction in growth rate with increasing depth (Bevan 1986). Although these studies support the hypothesis that the energetic cost of surfacing reduces the energy available for growth, they cannot be considered conclusive because of problems with water quality and limited replication. In the studies of *Channa*, *Heteropneustes*, and *Rana*, dissolved oxygen level was not controlled and, in most cases, was negatively correlated with water depth. Sample sizes of three to six in studies of *Channa*, *Heteropneustes*, and *R. tigrina* and pseudoreplication in studies of *R. pipiens* and *Clarias* further limit the evaluation of a depth effect.

In this study, we examined the effect of water depth on the distance travelled to breathe air and on the growth rate of *C. macrocephalus*. This species is commercially important in Asia (Panayotou et al. 1982) and is a continuous, facultative air breather, predominantly benthic and sedentary in habit (Bevan and Kramer 1987).

Materials and Methods

Clarias macrocephalus fry (full siblings) were obtained from a wild stock originating in Perak, Malaysia. They were initially fed live *Artemia salina* nauplii, and later, sinking trout feed (GRT-83G: Martin Feed Mills, Ontario, Canada). Fish were maintained under a 12L:12D photoperiod and 27°C

TABLE 1. Effect of depth on the surfacing activity and growth of *C. macrocephalus*. (A) Feeding experiment, 28 d; sample size = 12, except for 118 cm (N = 11). (B) Fasting experiment, 14 d; sample size = 12 (except for 30 cm (N = 11). Data are mean ± SE

Variable	Depth (cm)			
	30	60	90	118
	A. Feeding experiment			
Dissolved oxygen ($mg\ O_2 \cdot L^{-1}$)	0.84 ± 0.02	0.82 ± 0.02	0.69 ± 0.02	0.54 ± 0.03
Air-breathing frequency ($breaths \cdot h^{-1}$)	25.87 ± 4.81	25.10 ± 3.21	20.50 ± 1.40	21.68 ± 1.62
Distance travelled ($m \cdot h^{-1}$)	15.52 ± 2.89	30.12 ± 3.85	36.91 ± 2.53	51.16 ± 3.83
Initial weight (g)	4.56 ± 0.24	4.68 ± 0.23	4.40 ± 0.23	4.79 ± 0.29
Final weight (g)	12.11 ± 0.68	12.97 ± 0.60	12.64 ± 0.60	13.03 ± 0.65
Specific growth rate ($\% \cdot d^{-1}$)	3.47 ± 0.15	3.64 ± 0.17	3.78 ± 0.11	3.60 ± 0.18
	B. Fasting experiment			
Dissolved oxygen ($mg\ O_2 \cdot L^{-1}$)	0.18 ± 0.01	0.15 ± 0.00	0.15 ± 0.00	0.14 ± 0.00
Air-breathing frequency ($breaths \cdot h^{-1}$)	10.95 ± 1.16	8.62 ± 0.62	9.45 ± 0.67	9.60 ± 1.31
Distance travelled ($m \cdot h^{-1}$)	6.57 ± 0.70	10.34 ± 0.74	17.02 ± 1.21	22.66 ± 3.08
Initial weight (g)	12.44 ± 0.45	12.22 ± 0.56	12.66 ± 0.73	13.37 ± 0.70
Final weight (g)	11.25 ± 0.43	11.10 ± 0.53	11.49 ± 0.64	12.12 ± 0.61
Specific growth rate ($\% \cdot d^{-1}$)	−0.72 ± 0.03	−0.69 ± 0.02	−0.68 ± 0.03	−0.70 ± 0.03

(range 26–28°C). Prior to the first experiment, fish were acclimated to hypoxia (2 mg $O_2 \cdot L^{-1}$) for 21 d.

Forty-eight cages (15 cm diameter × 30, 60, 90, or 118 cm depth) were suspended, in a randomized block design, in two culture tanks. The depth range was chosen to approximate those of intensive culture conditions. Homogeneity of water, within and among cages, was achieved by recirculating the water through a deoxygenation tank, a header tank, and the two culture tanks (total volume 2100 L, turnover time 1.75 h). Water quality was maintained using four biological filters (1000 $L \cdot h^{-1}$) and replacing 15% of the water weekly. A Yellow Springs Instrument (model 57) oxygen meter continuously monitored dissolved oxygen in the header tank and was coupled to a solenoid valve regulating nitrogen gas flow to the deoxygenation tank. Dissolved oxygen and temperature within cages were checked twice weekly. Calibration of the meter was checked by the Winkler method and found accurate to within 0.1 mg $O_2 \cdot L^{-1}$. Further details of the experimental system are presented by Bevan (1986).

The distance travelled to breathe air was calculated from the surfacing frequency, assuming that each surfacing involved a journey of twice the maximum depth. Fish were weighed (±1 mg) and measured (±1 mm) weekly, and specific growth rate was calculated (Knights 1985). Because of slight differences in dissolved oxygen among treatments, the effect of depth on the specific growth rate was examined by analysis of covariance, with dissolved oxygen as the covariable (Sokal and Rohlf 1981). The data are presented as mean ± standard error, unless otherwise indicated.

A. Feeding experiment — Forty-eight fish (2.93–6.60 g, 7.3–9.2 cm) were weighed, measured, and randomly assigned to individual cages (12 fish per depth of 30, 60, 90, and 118 cm). Fish were fed 6% of the mean body weight daily (N = 48), in two equal amounts, for 28 d. The ration was recalculated after each weekly weighing. Dissolved oxygen level within cages was maintained at 0.72 ± 0.02 mg $O_2 \cdot L^{-1}$. This level, which is similar to the minimal levels observed in intensive *Clarias* rearing ponds (Bok and Jongbloed 1984), was selected to increase the air-breathing frequency and therefore the variation among groups of fish in the distance travelled to breathe (Bevan and Kramer 1987). For each fish, 12 observations of air-breathing frequency were made (30-min duration each, three per week).

B. Fasting experiment — On termination of the feeding experiment, 9 of the 12 fish from each original depth were randomly reassigned to a new depth treatment and 3 were maintained at the same depth; all fish were randomly reassigned to a new cage location. The fish were then maintained for a further 14 d without feeding, at 0.15 ± 0.003 mg $O_2 \cdot L^{-1}$. For each fish, six observations of air-breathing frequency were made.

Results

A. Feeding experiment — The distance travelled to breathe air increased strongly with depth, averaging 15.5 $m \cdot h^{-1}$ (150 $TL \cdot h^{-1}$) at 30 cm and 51.5 $m \cdot h^{-1}$ (492 $TL \cdot h^{-1}$) at 118 cm (Table 1A). The initial mean weights of fish in each treatment were not significantly different (F = 0.39, P = 0.76, ANOVA), and all fish increased in weight (7.91 ± 0.29 g). However, for one fish (118 cm depth) the individual weight gain was 0.06 g (specific growth rate $0.05\% \cdot d^{-1}$), and this clear outlier was omitted from all data analysis. The mean specific growth rate increased from $3.47\% \cdot d^{-1}$ at 30 cm to $3.78\% \cdot d^{-1}$ at 90 cm (Table 1A). There was no effect of block

TABLE 2. Analysis of covariance for the effect of depth on the specific growth rate of *C. macrocephalus*, with dissolved oxygen level as the covariate.

Source of variation	df	SS	MS	*F*	*P*
		A. Feeding experiment			
Adjusted means	3	1.6757	0.5586	2.23	0.10
Covariate	1	1.2323	1.2323	4.93	0.03
Error	42	10.4999	0.2500		
		B. Fasting experiment			
Adjusted means	3	0.0057	0.0019	0.18	0.91
Covariate	1	0.0186	0.0186	1.74	0.19
Error	42	0.0107			

on growth rate ($F = 0.96, P = 0.45$, ANOVA), and therefore the data were combined. Analysis of covariance, with dissolved oxygen as the covariable, showed that water depth had no significant effect upon specific growth rate (Table 2A), averaging $3.62 \pm 0.08\% \cdot d^{-1}$ ($N = 47$). Comparison of the least squares estimates showed that the mean specific growth rate at 30 cm was significantly lower than that at 90 and 118 cm ($P = 0.02$ and 0.03, respectively, *t*-test).

B. Fasting experiment — The mean distance travelled to breathe air ranged from $6.6\ m \cdot h^{-1}$ ($57\ TL \cdot h^{-1}$) at 30 cm to $22.7\ m \cdot h^{-1}$ ($188\ TL \cdot h^{-1}$) at 118 cm (Table 1B). The initial mean weights of fish in each treatment were not significantly different ($F = 0.96, P = 0.42$, ANOVA), and all fish lost weight (-1.18 ± 0.04 g). Analysis of covariance, with dissolved oxygen as the covariable, showed that water depth had no significant effect upon specific growth rate (Table 2B), averaging $-0.70 \pm 0.01\% \cdot d^{-1}$ ($N = 47$).

Discussion

For a benthic and normally sedentary fish like *Clarias*, travel to and from the surface is a large proportion of the total swimming activity, with its magnitude depending upon air-breathing frequency, water depth, and swimming velocity. Bevan and Kramer (1987) measured the swimming velocity of *C. macrocephalus* (12.7 g, 11.7 cm TL) as $2.9\ TL \cdot s^{-1}$ in cages similar to those in the present study. Assuming a similar velocity here, the temporal cost of surfacing increased from $0.9\ min \cdot h^{-1}$ (1.6%) at 30 cm to $3.1\ min \cdot h^{-1}$ (5.1%) at 118 cm. Although these are probably underestimates, since *Clarias* are more active at night (Jordan 1976, Bevan 1986), they are nonetheless an order of magnitude lower than the $11.4\ min \cdot h^{-1}$ (19%) estimated for *Channa striatus* at only 40 cm (Pandian and Vivekanandan 1976).

Previous studies of air-breathing fishes and amphibians have suggested that the energetic cost of surfacing from greater depths can be large enough to reduce growth. Our study clearly demonstrates that increased depth does not reduce the growth rate of juvenile *C. macrocephalus*. In addition, the observed trend of growth rate increasing with depth is directly opposite to that predicted. The experimental conditions of our study were such that if a strong depth effect on the growth of *Clarias* exists, then it should have been apparent. The maximum depth approaches the 1–2 m of commercial *Clarias* ponds, and the 6% ration is comparable with intensive practices (Panayotou et al. 1982). Furthermore, the proportion of activity that was dependent upon depth was increased in our study because of the low level of dissolved oxygen and the way in which the cages constrained horizontal swimming.

Potentially the differences in the energetic costs of surfacing were too small to be detected by our measurement of wet weight. If a large difference in proximate body composition had existed at the end of the feeding experiment, the reallocation for the fasting period would have increased the variance of specific growth rate among depths; this was not found. While not suggesting that the energetic cost of swimming is trivial (Priede 1985), it appears that under aquacultural conditions the cost of surfacing would be a very small component of the energy budget of juvenile *C. macrocephalus*.

Our study also shows that *C. macrocephalus* can achieve moderately high growth rates under severe hypoxia. However, the average specific growth rate of $3.6 \pm 0.08\% \cdot d^{-1}$ at 0.7 mg $O_2 \cdot L^{-1}$ is significantly lower than the $4.4\% \cdot d^{-1}$ achieved at normoxia (D. J. Bevan and D. L. Kramer, unpubl. data), and relatively small differences in dissolved oxygen did have a significant effect on growth in the present study. Previous studies suggesting that growth of air-breathing fishes is independent of dissolved oxygen have not examined such extreme hypoxia as that in our study, e.g. 1.2 mg $O_2 \cdot L^{-1}$ (Pandian et al. 1977) and 2.5 mg $O_2 \cdot L^{-1}$ (Jana and Das 1980). The reduction in growth rate and increased dependence upon dissolved oxygen by juveniles (Vivekanandan 1977a; Babiker 1984) suggest that dissolved oxygen levels in the intensive culture of air-breathing fishes, especially juveniles, may be important.

Finally, behavioural responses to stress have been generally overlooked in aquaculture (Knights 1985). The present study, and others, have observed that very shallow depths tend to reduce growth (Pandian and Marian 1985; Bevan 1986) and to increase mortality due to stress (Kolbeinshavn and Wallace 1985) and predation (Power 1986). In addition, several parameters of water quality (e.g. temperature stability) are strongly correlated with depth. We suggest that the influence of depth on behaviour, water quality, and predation deserves more serious attention in the development of aquaculture.

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Aggregation in Littoral Amphipod Populations: Transformation Controversies Revisited

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France, R. L. 1987. Aggregation in littoral amphipod populations: transformation controversies revisited. Can. J. Fish. Aquat. Sci. 44: 1510–1515.

Spatial variability in amphipod (*Hyalella azteca*) density was examined in 17 Canadian Shield Lakes in south-central Ontario. Aggregation was measured by the exponent of the power relationship between density and variance. The average *b* index was 1.45, although this value varied depending on lake and habitat type. Whereas this should indicate the use of a fourth-root transformation, neither this nor the logarithmic transformation was as effective as the square-root in stabilizing variance. A literature review suggests that if a universal transformation is sought for benthos data, it should not be the commonly used log transformation. The observation that the square-root transformation seemed to work better on average than the fourth-root transformation, even in the region suggested by theory for the latter, suggests that complications may arise in the uncritical use of Taylor's Power Law.

On a étudié la variabilité spatiale de l'amphipode (*Hyalella azteca*) dans 17 lacs du Bouclier canadien situés dans la partie sud centrale de l'Ontario. Le regroupement des amphipodes est représenté par l'exposant dans la relation de puissance entre la densité et la variance. L'indice *b* moyen se situe à 1,45 mais cette valeur variait selon le lac et le type d'habitat. Alors que ceci devrait indiquer l'utilisation d'une transformation de la quatrième racine, ni celle-ci ni la transformation logarithmique ne se sont révélées aussi efficace que la racine carrée pour stabiliser la variance. Une étude documentaire porte à croire que la transformation logarithmique communément utilisée ne devrait pas servir de transformation universelle des données sur le benthos. Le fait que la transformation de la racine carrée semble donner, en moyenne, de meilleurs résultats que la transformation de la quatrième racine suggère que des complications peuvent survenir à l'utilisation sans discernement de la loi de puissance de Taylor.

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Because the density distributions of many benthic species are aggregated (Gage and Geekie 1973; Resh 1979; Downing 1979), data must be subjected to some form of transformation, usually to stabilize the variance (i.e. eliminate the correlation between the variance and the mean), prior to parametric statistical analysis (Elliott 1979). Transformation to stabilize the variance should also alleviate skewness in the distribution as well as increasing the probability of variance additivity. Both of these are key assumptions for analysis of variance and linear regression (Downing 1979).

The choice of a particular transformation is dependent on the variance to mean ratio, and as such is a function of the organism's distribution (Taylor 1961). Much debate has involved the question of whether specificity or catholicism is the most sought after attribute in seeking a transformation. W. D. Taylor (1980) maintained that because benthic populations vary greatly in degree of aggregation, "each data set should receive individual appraisal and treatment." However, despite most researchers recognizing the limitations for a single transformation to account for all the residual variation in particular cases (e.g. Morin 1985), many may accept this loss of precision for overall generality (Downing 1980). This is thought justified because of the reported homogeneity in aggregation among different animals, samplers, and substrates (Downing 1979). There is nevertheless no general agreement on the most suitable universal transformation.

Green (1979) assumed that in most cases it makes sense to choose either the square-root or logarithmic transformation "rather than something in between." This is the view first expressed for benthos data by Elliott (1979). Downing (1979, 1980, 1981), on the other hand, showed that the log transformation was on average too extreme in that it tended to overtransform benthos data, while the square-root transformation undertransformed data. He suggested that a compromise, the fourth-root transformation, was the most useful to apply routinely. Alternatively, Chang and Winnell (1981) found that the conventional log transformation was as adept at uncorrelating means and variances as was Downing's fourth-root transformation. Based on this, they suggested that "restraint should be placed on the use of this [the fourth-root] transformation for benthic data." Finally, L. R. Taylor (1980) reviewed the debate in light of his own publications on terrestrial insects and concluded diplomatically that perhaps everyone had a valid argument: "I agree with W. Taylor's main point, that a common transformation is unlikely to be adequate for all benthos species... Downing may be right, if any transformation is feasible, to suggest the fourth-root when the taxa are large and the data limited. In contrast, analysis of detailed or extensive sampling for a single species will require a specific transformation, while the *ad hoc* choice of square root and log may well remain the most convenient compromise."

Although there are some data on densities of littoral macrobenthos in Canadian Shield lakes (Hamilton 1971; Allison and Harvey 1981; Dermott 1985), variability about these estimates has not been given. Collins et al. (1981) did find, however, the log transformation sufficient to normalize total species estimates of biomass in Ontario softwater lakes. The objective of the present analysis was to determine which of the three suggested "universal" transformations (square-root, fourth-root, or logarithmic) can be best used routinely to produce the least correlation between variances and means of density estimates for *Hyalella azteca* (Saussure), the most ubiquitous amphipod in Canadian lakes (Bousfield 1958). As well, the range of aggregation within a species may vary depending on habitat variability. Examination of this relationship may offer insight into the balance between environmental influences and the large-scale consistency attributed to innate biological factors of individual species (Taylor et al. 1980) as co-determinants of spatial distribution.

Methods

I sampled *H. azteca* populations from 17 lakes in the Haliburton–Muskoka region of south-central Ontario during the ice-free months (May to November) of 1984 and 1985. Morphometric and chemical data for many of these lakes are available in Yan et al. (1985). Population densities and reproductive behavior of *H. azteca* have been described by Stephenson and Mackie (1986) and France (1987). Amphipods were sampled from three representative habitats within each lake: (1) areas of maximum plant growth, usually located just off the mouths of inflows and representing on average 13% (range = 4–42%) of the shoreline length; (2) exposed sandy substrates with sparse macrophytic cover, representing about 30% (range = 10–62%) of the shoreline length; and (3) predominately allochthonous detritus/organic sediments comprising 33% (range = 10–44%) of the shoreline length.

The sampling apparatus was a circular corer (7.62 cm diameter, 45.6, cm^2) of PVC pipe which could be forced into the substrate with a handle (Stephenson and Mackie 1986; France 1987). All cores were taken from between 0.1 and 0.7 m depth and always penetrated at least 5 cm below the substrate surface. The range of estimated mean density was from 44 to $6242 \cdot m^{-2}$, sample variance from 5.4×10^3 to 7.4×10^7, sample number from 5 to 13, and sampling frequency from 2 to 14 per lake. In total, 149 paired observations of $\bar{X}$ and s^2 from true replicates (same date/same station) were used for analysis.

Analysis — Taylor's Power Law (Taylor 1961) is the most effective description of the density dependence of population variance (Taylor et al. 1980). The slope of the relationship between $\log s^2$ and $\log \bar{X}$ density is b, the index of aggregation. The larger the value of b, the greater the degree of aggregation within the population (i.e. $s^2 >> \bar{X}$); $a = 1$, $b = 1$ is the special case of the random or Poisson distribution ($s^2 = \bar{X}$), while $b = 2$ indicates a log-normal distribution (Elliott 1979; Downing 1979). The exact variance stabilizing function can be calculated once the index of aggregation is known (Taylor 1961):

$$(1) \quad X' = X^{1-(b/2)}$$

where X is density per square metre, b is the power function exponent, and X' is the transformed datum.

The best single transformation to apply routinely is the one which usually yields the least correlation between variances and means (Downing 1981). Following the examples of Downing (1981) and Chang and Winnell (1981), I performed the square-root ($b = 1$, $1 - b/2 = 0.5$), fourth-root ($b = 1.5$, $1 - b/2 = 0.25$), and log ($b = 2$, $1 - b/2 = 0$) transformations on the amphipod data broken down on either a per lake or per habitat basis. I then examined plots of the coefficient of determination (r^2) of variance to mean regressions after transformation, over the range of observed population aggregation (b values).

TABLE 1. Coefficients of determination (r^2) of the relationship between $\log_{10} s^2$ and $\log_{10} \bar{X}$ for untransformed data and data transformed with the square-root ($X^{0.5}$), fourth-root ($X^{0.25}$), and logarithmic ($\ln(X + 1)$) transformations. An asterisk indicates that there is a significant ($P < 0.05$) correlation between s^2 and $\bar{X}$. The ranks of the coefficient when compared with coefficients obtained using other transformations are shown. The slope of the log−log relationship between s^2 and $\bar{X}$ before transformation is denoted as b, and the exact variance stabilizing function is calculated as $1 - b/2$. The best transformation to use routinely is that which shows the lowest r^2 or has the lowest average rank.

	Untransformed				r^2 (rank $\|\|r^2\|\|$)		
Division	n	r^2	b	$1 - b/2$	$X^{0.5}$	$X^{0.25}$	$\ln(X + 1)$
Blue Chalk	33	0.77*	1.30	0.35	0.07 (1)	0.24 (2)	0.39* (3)
Red Chalk Main	19	0.64*	1.55	0.23	0.32 (2)	0.30 (1)	0.43 (3)
Red Chalk Bay	15	0.54*	1.18	0.41	0.04 (1)	0.31 (2)	0.34 (3)
Little Clear	19	0.76*	1.61	0.20	0.18 (1)	0.30 (2)	0.68* (3)
Gullfeather	16	0.82*	1.50	0.25	0.15 (1)	0.50* (2)	0.62* (3)
Heaney	17	0.78*	1.37	0.32	0.07 (1)	0.30 (2)	0.48 (3)
Dickie	21	0.84*	1.41	0.30	0.31 (2)	0.06 (1)	0.52* (3)
Glen	19	0.35	1.00	0.50	0.04 (1)	0.39 (2)	0.76* (3)
"B" lakes[a]	19	0.73*	1.52	0.24	0.12 (1)	0.58* (2)	0.76* (3)
Macrophytes	99	0.67*	1.57	0.22	0.09 (1)	0.23* (2)	0.34* (3)
Sandy	33	0.52*	1.09	0.46	0.06 (1)	0.30 (2)	0.38* (3)
Detritus	35	0.46*	1.14	0.43	0.02 (1)	0.28 (2)	0.29 (3)
Total	159	0.76*	1.45	0.28	0.11 (1)	0.18* (2)	0.40* (3)
Sum of ranks					(15)	(24)	(39)

[a] Crosson, Harp, Healy, Leech, Bentshoe, Leonard, McKay, Solitare, Chub.

Results and Discussion

Aggregation — Populations of *H. azteca* in Canadian Shield lakes are aggregated with an average b of 1.45 (Table 1). This is similar to the value of 1.49 determined for *Pontoporeia hoyi* from nearshore regions of Lake Michigan (Chang and Winnell 1981), as well as the average b of 1.34 derived for amphipods in general (Downing 1979). Other benthic organisms such as water mites and nematodes were more aggregated ($b \approx 1.75$); based on worldwide data, amphipods are ranked only sixth out of 15 different benthic taxa in degree of contagion (Downing 1979).

Although regressions of log variance on log mean density do yield stable coefficients reflecting population parameters, W. D. Taylor (1980) cautioned that the specificity of b depends on the density-dependent interaction with the environment. Therefore, whereas b remains consistent for a given organism and environment, fine-scale environmental changes can cause b to range considerably for some species. These behavioural changes are thought to be influenced by habitat structure, resource distribution, or life cycle seasonality (Taylor et al. 1980). The aggregation index b varied from 1.00 to 1.61 for *H. azteca* populations among study lakes (Table 1). Intercepts of regression equations were always greater than the log of the constant (219) used to convert whole numbers to density per square metre (W. D. Taylor, pers. comm.), so that even in the case of Glen Lake the amphipods cannot be considered randomly distributed. None of the relationships were curvilinear, in contrast with what Morin (1985) found for stream benthos. The b values did not correlate with densities (Spearman's rank test; $P > 0.05$). It is conceivable that lake differences in b may reflect differing proportions of the three representative habitats specific to each lake, as these seem to have a marked influence on aggregation (Table 1).

It is also interesting to note that amphipods are more uniformly distributed over macrophytes ($b = 1.57$) than the physical clumping of the environment itself would suggest (i.e. the variance function for estimation of macrophyte biomass obtained from these same cores has a power exponent of 2.00; R. L. France, unpubl. data). This may imply the operation of some form of density-dependent regulation of amphipod distribution.

Transformation — The coefficients of determination (r^2) for log−log regressions between the variances and means of untransformed and transformed data are shown in Table 1. With the exception of Glen Lake, all regressions. Loss of significance after transformation indicates that particular transformation was successful in stabilizing variance. The square-root transformation always stabilized the variance, while the log and fourth-root transformations recommended by Elliott (1979) and Downing (1979), respectively, failed in numerous cases. Further, a ranking analysis demonstrated that the coefficients of determination after log transformation were invariably the highest, while those after square-root transformation were almost always the lowest. The most efficient of these three transformations to routinely use is that which yields the lowest sum of ranks (Downing 1981); in the present case, this is the square-root transformation.

The effectiveness of the three transformations in relation to the index of aggregation is shown in Fig. 1. The closer the coefficients of determination to zero, the more efficient the variance stabilizing function of that transformation. Both the log, and to a lesser degree the fourth-root transformation, were too extreme and tended to overtransform the data. The square-root transformation worked ideally to b values of about 1.35; above this, data were undertransformed. Interestingly, the square-root transformation yielded smaller r^2 values than the fourth-root transformation even in the region suggested by theory for the latter (i.e. $b \approx 1.5$; Downing 1981). Theoretically, the exact transformation to apply routinely for *H. azteca* data should be a compromise between these latter two, such as,

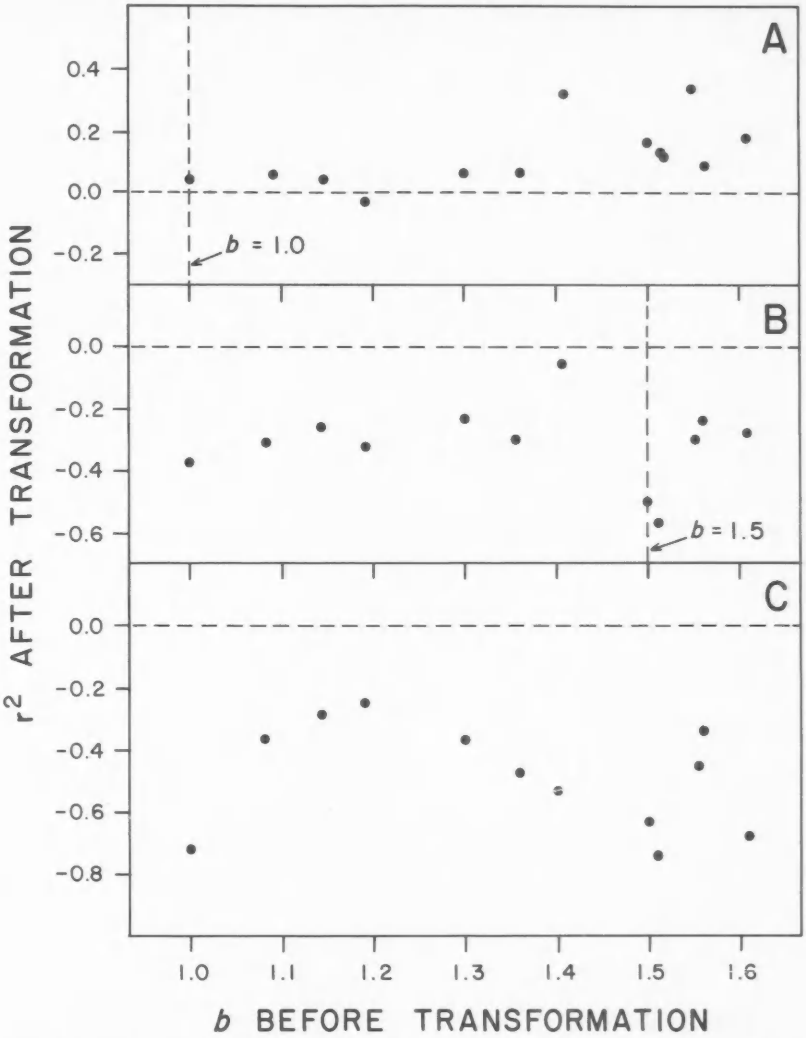

FIG. 1. Comparisons of three transformations for the stabilization of amphipod data grouped as in Table 1. The coefficents of determination (r^2, sign of slope considered) for the log−log regression of s^2 on $\bar{X}$ density (transformed data) are plotted against the slope of the initial relationship before transformation as in Downing (1981). The transformations are the (A) square-root, (B) fourth-root, and (C) logarithmic.

perhaps, the third-root. Unjustified *ad hoc* use of the log transformation for *H. azteca* should be avoided if further statistical analysis is planned.

A universal transformation? — Despite recognition that a common transformation may not only be inadequate for all benthos species, but that the naive use of one could actually be hazardous (W. D. Taylor 1980; Downing 1981), Downing (1981) *is* correct in his assertion that it is most common for researchers to use transformations "consistently without checking to see whether they are appropriate." The question remains as to which of the three basic transformations, square-root, fourth-root, or logarithmic, operates most effectively if chosen solely a *priori*.

Downing's selection of an intermediate transformation seems justified since Taylor et al. (1978) found that the frequency distribution of *b* from 156 data sets (including annelids, molluscs, crustaceans, insects, birds, fish, and orchids) was 1.45 ± 0.39 (SD). This is close to the theoretical optimum of 1.5 for maximum success with the fourth-root transformation and may explain why fully one quarter of the regressions of variance on mean for insects remained highly significant *after* log transformation of original data (Taylor and Woiwood 1980).

I combined my data with those from Downing (1979), Chang and Winnell (1981), and Downing (1981) and calcu-

TABLE 2. Evaluation of effectiveness of the square-root ($X^{0.5}$), fourth-root ($X^{0.25}$), and logarithmic ($\ln(X + 1)$) transformations in alleviating correlation between s^2 and $\bar{X}$ for untransformed (UT) data. N denotes number of individual cases examined per study, n indicates number of paired observations of s^2 and $\bar{X}$ per case, and b represents the power function exponent. The best overall transformation to consistently use is that which usually has the lowest number of significant coefficients of determination (r^2), the highest number of smallest ranked r^2, or the lowest average sum of ranks.

				Significant r^2				Number of smallest r^2			Sum of ranks		
Source	N	n	b	UT	$X^{0.5}$	$X^{0.25}$	$\ln(X + 1)$	$X^{0.5}$	$X^{0.25}$	$\ln(X + 1)$	$X^{0.5}$	$X^{0.25}$	$\ln(X + 1)$
Downing 1979	7	6–18	1.18–2.36	7	2	0	1	3	1	3	15	14	13
Chang and Winnell 1981	5	20	1.49–3.75	5	3	4	3	1	1	3	12	9	9
Downing 1981	31	4–12	−1.67–4.97	19	4	6	8	14	9	8	60.5	53.5[a]	72
This study	13	16–159	1.00–1.61	12	0	4	9	11	2	0	15	24	39
Total				44	9	14	21	29	13	14	102.5	100.5	133.0

[a] Value corrected for a slight mistake in table 1 in Downing (1981).

lated the average aggregation index to be 1.66 ± 0.93. The conclusion one would therefore reach was that Downing's fourth-root transformation should be the most universally acceptable of the three. However, as the *H. azteca* data showed, it is misleading to assume that the fourth-root transformation will invariably alleviate correlation between the mean and variance when b approximates 1.5 (e.g. Gullfeather Lake, "B" lakes, and macrophytes in Table 1). This is not unique to amphipods. Data from all four studies showed that there were 20 aggregation indices between 1.25 and 1.75. In only six of these did the theoretically optimal fourth-root transformation perform better than the square-root transformation in stabilizing variance. In fact, it actually failed to uncorrelate means and variances in six cases where the square-root proved effective (e.g. April 1977, Chironomidae, $b = 1.50$ (Table 1 in Chang and Winnell 1981); 1971, Nb-2, total, $b = 1.71$ (Table 1 in Downing 1981)). Complications may arise in the precision of Taylor's Power Law depending on the relative predominance of low and especially zero density counts (P. F. Olesiuk, Pacific Biological Station, Nanaimo, B.C., pers. comm.). Further, the dependence of s^2 on $\bar{X}$ in some benthic data sets (Gage and Geekie 1973) may confound estimation of degree of contagion as well as invalidating comparisons of distribution made among different communities in some situations.

A comparison of the four studies examining the relative merit of the three common transformation procedures revealed the following (Table 2): (1) for much benthos abundance data, the log transformation is too severe and as a result could frequently hinder rather than help statistical analysis; (2) the square-root is more successful than the fourth-root transformation in uncorrelating means and variances and has the slight edge in terms of absolutely diminishing coefficients of determination.

If any single transformation is to be indiscriminantly applied to benthos data, I agree with Downing's (1981) conclusion that the more central fourth-root is preferable to the logarithmic transformation, but I do not accept his belief in its general superiority over the square-root transformation. Perhaps, as W. D. Taylor (1980) suggested, the safest approach would be to calculate the precise transformation specific to each data set. However, the indication from this as well as previous studies, that the superiority of the square-root over the fourth-root transformation may even extend to b values exceeding 1.5, is alarming in terms of the rationale for selecting transformations to begin with. More thorough analysis, possibly through detailed simulations on derived data, should be undertaken to examine the circumstances in which the Power Law may fail to operate as optimally as its theoretical basis would suggest.

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CORRECTIONS TO RECENT PUBLICATIONS
CORRECTIONS SUR RECENTES PUBLICATIONS

VOLUME 42, No. 9 — article by L. Bernatchez and J. J. Dodson: *Influence of temperature and current speed on the swimming capacity of lake whitefish* (Coregonus clupeaformis) *and cisco* (C. artedii).

Page 1525: Table 3 values for standard metabolism, active metabolism, and metabolic scope for activity with 95% confidence intervals for *C. artedii* are incorrect due to an error in calculation. These should read $39 < 49 < 63 <$; $332 < 367 < 406 <$; and $293 < 318 < 343$.

Page 1527: Values in Table 5 for *C. artedii* and *C. clupeaformis* were obtained from the oxygen consumption – swimming speed regressions developed in the study and not from the exponent of 0.8 (Basu 1959), as indicated in the legend.

VOLUME 43, No. 11 — article by J. F. Caddy: *Modelling stock-recruitment processes in Crustacea: some practical and theoretical perspectives.*

Page 2332: Figure 1 calculations should reflect that only female recruits are being considered: assuming a 50:50 sex ratio, numbers at 6 yr, 9 mo should be divided by 2. This of course affects estimated population change.

Page 2339, left column: Line 6 should read "...with two values of D from 1.05 to 1.79..." and line 9 should read "...at around 3 cm (point B)..."

Page 2340: The two lines in Fig. 6 should be labelled $D = 1.79$ and $D = 1.05$ and the abscissa should be labelled 10 and 100 mm.

VOLUME 44, No. 2 — article by J. D. Rodgers, R. D. Ewing, and J. D. Hall: *Physiological changes during seaward migration of wild juvenile coho salmon* (Oncorhynchus kisutch).

Pages 454 and 455: Due to a production error, a portion of the first paragraph under the Discussion on page

454 was inadvertantly transposed to page 455, left column and first seven lines of the right column.

CAN. SPEC. PUBL. FISH. AQUAT. SCI. 89 — article by B. D. Glebe and R. L. Saunders: *Genetic factors in sexual maturity of cultured Atlantic salmon* (Salmo salar) *parr and adults reared in sea cages.*

Page 26: In the legend to Table 2, the equation for heterosis should read as follows:

$$\frac{(B \times R) + (R \times B)}{2} - \frac{(B \times B) + (R \times R)}{2}.$$

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